WO 97/42222 (Cyclacel Ltd) discloses peptide fragments of $p21^{WAF1}$ that interact with CDK4/cyclin D1. Thus it was observed that $p21_{(16-35)}$ and $p21_{(46-65)}$ bind to CDK4 and cyclin D1 respectively. Of these, only $p21_{(16-35)}$ was observed to inhibit CDK activity. $p21_{(141-160)}$ was observed to bind to CDK4 and cyclin D1 and to be a potent inhibitor of CDK4.

This data supported the known phenomenon of peptides including the sequence LFG as being the binding motif essential for the interaction of the p21 family with cyclins [Chen J et al. (1996), Lin J et al. and Russo AA et al.] and the further known properties of the amino-terminal half of p21 as being required for binding to CDK complex.

It should be borne in mind when considering the prior art discussed herein that unless otherwise explicitly stated the references to "motifs" is made with reference to papers that have made deductions and predictions based upon the activity of longer peptides usually consisting of at least 12 amino acids. Thus, the motifs are no more than conjecture based upon the specific set of reactions. Such motifs provide no indication as to the actual length of peptide or modifications that could be made to retain and/or even enhance activity or specificity.

The sequence $p21_{(141-160)}$ (disclosed in WO97/42222 and Ball K. et al) in respect of cyclin D1/CDK4 inhibition was subjected to analysis in order to determine the minimum length of an inhibitory peptide upon which novel antiproliferative drugs could be designed. Observations of CDK4/cyclin D1 inhibitory activity led to the identification of an inhibitory motif comprising RRLIF ($p21_{(155-159)}$) (SEQ ID No. 184), the bold residues being described as essential for activity and the underlined residue contributing towards inhibitory activity. Further observations in these disclosures include the retention of inhibitory activity against cyclin D1-CDK4 by the peptide KRRLIFSK ($p21_{(154-161)}$) (SEQ ID No. 185) albeit at a concentration 1000 times greater than the parent sequence $p21_{141-160}$ and that the substitution of aspartic acid at position 149 of $p21_{141-160}$ by alanine surprisingly reduced the IC_{50} of the full length peptide from 100 nM to 46 nM. Thus, although identifying the RRLIF (SEQ ID No. 184) motif as being important to

cyclinD1/CDK4 inhibition, Ball et al. is inconclusive as to the actual minimum length peptide required for enhanced activity. The effect of the Asp149 to Ala substitution has not proven reproducible.

In summary, WO97/42222 and Ball et al teach that there are sequences within the carboxy terminal region of p21 that are capable of interacting with CDK4/cyclin D in a manner that is inhibitory to CDK4 and further involves specific binding to cyclin D. Though the peptide $p21_{(141-160)}$ is described as being preferred, an 8-mer comprising $p21_{(154-161)}$ (KRRLIFSK) (SEQ ID No. 185) was inhibitory, but at higher concentrations. Finally, alanine replacement at position 149 within $p21_{141-160}$ increased the inhibitory activity. Thus, although the art indicates that this is an interesting region of p21 to investigate, no guidance is provided as to the identity of further fragments that would be preferably active against CDK4/cyclin D or any other CDK/cyclin enzymes.

Chen J et al. (Mol Cell Biol (1996) 16(9) 4673-4682) discloses a 12-mer corresponding to $p21_{17-24}$ as being a cyclin binding domain of p21. They further identify a less avid cyclin binding region as $p21_{150-161}$. Mutation and inhibition analysis demonstrated that the principal site of interaction with cyclin A was $p21_{17-24,}$ being a better inhibitor than $p21_{150-161}$ consistent with its greater avidity for cyclins such that it can be detected by pull-down assay. Interaction of $p21_{150-161}$ could only "be inferred from competition for binding and kinase inhibition assays. The importance of the $p21_{150-161}$ *in vivo* was questioned due to the possibility of the relevant site being occupied by PCNA.

Adams DA et al. (Mol Cell Biol (1996) 16(12) 6623-6633) discloses N- and C-terminal regions of p21 that putatively bind to CDK2/cyclin. A 14-mer ($p21_{149-162}$) is disclosed as inhibiting the binding of cyclin A to E2F1 and the binding of cyclins A and E to GST-p21. An amino acid sequence containing 8 amino acid residues (PVKRRLDL) (SEQ ID No. 186) derived from the transcription factor E2F1 was shown to bind to cyclin A/E-CDK2 complexes. An alanine scan of the 8-mer identified, on a qualitative level that certain modified forms of the peptide retained this activity. Noteworthy is that deletion or alanine replacement of either terminal

amino acid reduced or abolished the ability to compete with GST-E2F1 for cyclin A binding.

In a further paper, Adams DA et al. (Mol Cell Biol (1999) 19(2) 1068-1080) investigated the existence of an E2F1-like motif within pRB as a means to explain its interaction with cyclin A/CDK2. A single 10-mer, pRB869-878 was the shortest pRB derived peptide investigated.

In a subsequent paper, Chen et al. (Proc. Natn. Acad. Sci. (1999) 96, 4325-4329) disclosed two E2F1 derived 8-mers as possessing the ability to interact with the cyclin A/CDK2 complex, being PVKRRLFG (SEQ ID No. 187) and PVKRRLDL (SEQ ID No. 186). These peptides were tested in whole cell assays using membrane translocation carrier peptides HIV-TAT or Penetratin®.

Brown NR et al. (Nature Cell Biol. (1999) 1, 438-443) describes a crystal structure of the cyclin A3/phospho-CDK2 complex with an 11-mer derived from p107 including the RXLF motif. Of the 11-mer, the region RRLFGE was found to be within the binding region of cyclin A forming interactions with M210, I213, W217, E220, L253 and Q254.

An aim of the present invention has been to identify further peptides derived from p21 that retain or improve upon the inhibitory activities described in the art, particularly with regard to substrate specificity and peptide chain length as described in detail below.

In another embodiment, a peptide is selected from;

DFYHSKRRLIFS (SEQ ID No: 4),

TDFYHSKRRLIF (SEQ ID No: 5),

AFYHSKRRLIFS (SEQ ID No: 6),

DAYHSKRRLIFS (SEQ ID No: 7),

DFAHSKRRLIFS (SEQ ID No: 8),

DFYASKRRLIFS (SEQ ID No: 9),

DFYHAKRRLIFS (SEQ ID No: 10),

DFYHSARRLIFS (SEQ ID No: 11),

DFYHSKRALIFS (SEQ ID No: 12),

DFYHSKRRLAFS (SEQ ID No: 13),

DFYHSKRRLIFA (SEQ ID No: 14),

FYHSKRRLIFS (SEQ ID No: 15),

YHSKRRLIFS (SEQ ID No: 16),

HSKRRLIFS (SEQ ID No: 17),

DFYHSKRRLIF (SEQ ID No: 18),

FYHSKRRLIF (SEQ ID No: 19),

YHSKRRLIF (SEQ ID No: 20),

HSKRRLIF (SEQ ID No: 21),

S K R R L I F (SEQ ID No: 22),

K R R L I F (SEQ ID No: 23),

H-	Arg-	Leu-	Ile-	Phe	-NH2					(SEQ ID No: 24)
H-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2				(SEQ ID No: 25)
H-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2			(SEQ ID No: 26)
H-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2		(SEQ ID No: 27)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	(SEQ ID No: 28)
H-	Asn-	Leu-	Phe-	Gly	-NH2					(SEQ ID No: 29)
H-	Arg-	Asn-	Leu-	Phe-	Gly	-NH2				(SEQ ID No: 30)
H-	Abu-	Arg-	Asn-	Leu-	Phe-	Gly	-NH2			(SEQ ID No: 31)
H-	Ala-	Abu-	Arg-	Asn-	Leu-	Phe-	Gly	-NH2	and	(SEQ ID No: 32)
H-	Ser-	Ala-	Abu-	Arg-	Asn-	Leu-	Phe-	Gly	-NH2	(SEQ ID No: 33)

In still another aspect, the invention pertains to a peptide of formula II: $X_1X_2X_3RX_4LX_5F$ (formula II) (SEQ ID No. 2) wherein X_1, X_3, X_4 and X_5 may be any amino acid and X_2 is serine or alanine; and variants thereof.

In one embodiment, X_5 is selected from isoleucine and glycine.

In one embodiment, X_1 and X_4 are both basic amino acid residues and X_3 is a basic or polar residue.

In one embodiment, X is histidine and X is arginine, and X is lysine or cystein.

In another aspect the invention pertains to a peptide of formula: $X_1X_2X_3RX_4LX_5F$ (SEQ ID No. 2) wherein X_1, X_3, X_4 and X_5 may be any amino acid and X_2 is serine or alanine; and variants thereof, wherein the peptide is modified by at least one of a deletion,

aliphatic side chain, such as norleucine, norvaline, cyclohexylalanine (Cha), phenylalanine or 1-naphthylalanine (1Nal), (g)X_5 is isoleucine or an alternative natural or unnatural amino acid residue having a slightly larger aromatic or aliphatic side chain, such as norleucine, norvaline, cyclohexylalanine (Cha), phenylalanine or 1-naphthylalanine (1Nal), (h)phenylalanine is replaced with a natural or unnatural amino acid such as leucine, cyclohexylalanine (Cha), homophenylalanine (Hof), tyrosine, parafluorophenylalanine (pFPhe), meta-fluorophenylalanine (mFPhe), trptophan, 1-naphthylalanine (1Nal), 2-naphthylalanine (2Nal), biphenylalanine (Bip) or Tic), (i) X_5 and the terminal phenylalanine residue are reversed, or (j) the peptide is in cyclic form by the formation of a linkage between the side chain of X_4 and the C-terminus residue.

In one embodiment, X_2 is alanine.

In one embodiment, X_5 is isoleucine.

In another embodiment, a peptide is selected form the group consisting of:

H S K R R L I F (SEQ ID No: 34),

H A K R R L I F (SEQ ID No: 35),

H S K R R L F G (SEQ ID No: 36),

H A K R R L F G (SEQ ID No: 37),

K A C R R L F G (SEQ ID No: 38),

K A C R R L I F (SEQ ID No: 39),

	X1	X2	X3	R	X4	L	X5	F		
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	- NH2	(SEQ ID No: 55)
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	- NH2	(SEQ ID No: 40)
	H-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	- NH2	(SEQ ID No: 41)
H-	Pya-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	- NH2	(SEQ ID No: 42)
H-	Thi-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	- NH2	(SEQ ID No: 43)
H-	Hse-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	- NH2	(SEQ ID No: 44)

H- Phe- Ala- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 45)

H- Dab- Ala- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 46)

H- His- Gly- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 47)

H- His- Abu- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 48)

H- His- Nva- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 49)

H- His- Bug- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 50)

H- His- Val- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 51)

H- His- Ile- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 52)

H- His- Phg- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 53)

H- His- Phe- Lys- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 54)

H- His- Ala- Ala- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 56)

H- His- Ala- Nle- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 57)

H- His- Ala- Abu- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 58)

H- His- Ala- Leu- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 59)

H- His- Ala- Arg- Arg- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 60)

H- His- Ala- Lys- Ala- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 61)

H- His- Ala- Lys- Cit- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 62)

H- His- Ala- Lys- Hse- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 63)

H- His- Ala- Lys- His- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 64)

H- His- Ala- Lys- Nle- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 65)

H- His- Ala- Lys- Gln- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 66)

H- His- Ala- Lys- Lys- Arg- Leu- Ile- Phe - NH2 (SEQ ID No: 67)

H- His- Ala- Lys- Arg- Ala- Leu- Ile- Phe - NH2 (SEQ ID No: 68)

H- His- Ala- Lys- Arg- Asn- Leu- Ile- Phe - NH2 (SEQ ID No: 69)

H- His- Ala- Lys- Arg- Pro- Leu- Ile- Phe - NH2 (SEQ ID No: 70)

H- His- Ala- Lys- Arg- Ser- Leu- Ile- Phe - NH2 (SEQ ID No: 71)

H- His- Ala- Lys- Arg- Aib- Leu- Ile- Phe - NH2 (SEQ ID No: 72)

H-	His-	Ala-	Lys-	Arg-	Sar-	Leu-	Ile-	Phe	-	NH2	(SEQ ID No: 73)
H-	His-	Ala-	Lys-	Arg-	Cit-	Leu-	Ile-	Phe	-	NH2	(SEQ ID No: 74)
H-	His-	Ala-	Lys-	Arg-	Arg-	Ala-	Ile-	Phe	-	NH2	(SEQ ID No: 76)
H-	His-	Ala-	Lys-	Arg-	Arg-	leu-	Ile-	Phe	-	NH2	(SEQ ID No: 77)
H-	His-	Ala-	Lys-	Arg-	Arg-	Ile-	Ile-	Phe	-	NH2	(SEQ ID No:78)
H-	His-	Ala-	Lys-	Arg-	Arg-	Val-	Ile-	Phe	-	NH2	(SEQ ID No: 79)
H-	His-	Ala-	Lys-	Arg-	Arg-	Nle-	Ile-	Phe	-	NH2	(SEQ ID No: 80)
H-	His-	Ala-	Lys-	Arg-	Arg-	Nva-	Ile-	Phe	-	NH2	(SEQ ID No: 81)
H-	His-	Ala-	Lys-	Arg-	Arg-	Cha-	Ile-	Phe	-	NH2	(SEQ ID No: 82)
H-	His-	Ala-	Lys-	Arg-	Arg-	Phe-	Ile-	Phe	-	NH2	(SEQ ID No: 83)
H-	His-	Ala-	Lys-	Arg-	Arg-	1Nap-	Ile-	Phe	-	NH2	(SEQ ID No: 84)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ala-	Phe	-	NH2	(SEQ ID No: 85)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Leu-	Phe	-	NH2	(SEQ ID No: 86)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Val-	Phe	-	NH2	(SEQ ID No: 87)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Nle-	Phe	-	NH2	(SEQ ID No: 88)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Nva-	Phe	-	NH2	(SEQ ID No: 89)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Cha-	Phe	-	NH2	(SEQ ID No: 90)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Phe-	Phe	-	NH2	(SEQ ID No: 91)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	1Nap-	Phe	-	NH2	(SEQ ID No: 92)
	H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Phe	-	NH2	(SEQ ID No: 93)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Leu	-	NH2	(SEQ ID No: 95)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Cha	-	NH2	(SEQ ID No: 96)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Hof	-	NH2	(SEQ ID No: 97)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Tyr	-	NH2	(SEQ ID No: 98)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 99)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	mFPhe	-	NH2	(SEQ ID No: 100)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Trp	-	NH2	(SEQ ID No: 101)

H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	1Nap	-	NH2	(SEQ ID No: 102)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	2Nap	-	NH2	(SEQ ID No: 103)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Lys	-	NH2	(SEQ ID No: 104)
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Tic	-	NH2	(SEQ ID No: 105)
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	L-Pse		OH	(SEQ ID No: 106)
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	D-Pse		OH	(SEQ ID No: 107)
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	L-Pse		OH	(SEQ ID No: 108)
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	D-Pse		OH	(SEQ ID No: 109)
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	L-Psa		OH	(SEQ ID No: 110)
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	D-Psa		OH	(SEQ ID No: 111)
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	L-Psa		OH	(SEQ ID No: 112)
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	D-Psa		OH	(SEQ ID No: 113)
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	Dhp		OH	(SEQ ID No: 114)
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	Dhp		OH	(SEQ ID No: 115)
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	Pheol			(SEQ ID No: 116)
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	Pheol			(SEQ ID No: 117)
H-	Ala-	Ala-	Abu-	Arg-	Arg-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 118)
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 119)
H-	Ala-	Ala-	Lys-	Arg-	Cit-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 120)
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ala-	pFPhe	-	NH2	(SEQ ID No: 121)
H-	Ala-	Ala-	Abu-	Arg-	Ser-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 122)
H-	Ala-	Ala-	Lys-	Gln-	Arg-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 123)
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 124)
H-	Gly-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 125)
H-	Ala-	Ala-	Lys-	hArg-	Arg-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 126)
H-	Ala-	Ala-	Lys-	Ser-	Arg-	Leu-	Ile-	pFPhe	-	NH2	(SEQ ID No: 127)
H-	Ala-	Ala-	Lys-	Hse-	Arg-	Leu-	Ile-	pFPhe		NH2	(SEQ ID No: 128)

H- Ala- Ala- Lys- Arg- Lys- Leu- Ile- pFPhe - NH2 (SEQ ID No: 129)

H- Ala- Ala- Lys- Arg- Orn- Leu- Ile- pFPhe - NH2 (SEQ ID No: 130)

H- Ala- Ala- Lys- Arg- Gln- Leu- Ile- pFPhe - NH2 (SEQ ID No: 131)

H- Ala- Ala- Lys- Arg- Hse- Leu- Ile- pFPhe - NH2 (SEQ ID No: 132)

H- Ala- Ala- Lys- Arg- Thr- Leu- Ile- pFPhe - NH2 (SEQ ID No: 133)

H- Ala- Ala- Lys- Arg- Nva- Leu- Ile- pFPhe - NH2 (SEQ ID No: 134)

H- Ala- Ala- Lys- Arg- Arg- Phg- Ile- pFPhe - NH2 (SEQ ID No: 135)

H- Ala- Ala- Lys- Arg- Arg- Met- Ile- pFPhe - NH2 (SEQ ID No: 136)

H- Ala- Ala- Lys- Arg- Arg- Ala- Ile- pFPhe - NH2 (SEQ ID No: 137)

H- Ala- Ala- Lys- Arg- Arg- Hof- Ile- pFPhe - NH2 (SEQ ID No: 138)

H- Ala- Ala- Lys- Arg- Arg- hLeu- Ile- pFPhe - NH2 (SEQ ID No: 139)

H- Ala- Ala- Lys- Arg- Arg- aIle- Ile- pFPhe - NH2 (SEQ ID No: 140)

H- Ala- Ala- Lys- Arg- Arg- Leu- Gly- pFPhe - NH2 (SEQ ID No: 141)

H- Ala- Ala- Lys- Arg- Arg- Leu- βAla pFPhe - NH2 (SEQ ID No: 142)

H- Ala- Ala- Lys- Arg- Arg- Leu- Phg- pFPhe - NH2 (SEQ ID No: 143)

H- Ala- Ala- Lys- Arg- Arg- Leu- Aib- pFPhe - NH2 (SEQ ID No: 144)

H- Ala- Ala- Lys- Arg- Arg- Leu- Sar- pFPhe - NH2 (SEQ ID No: 145)

H- Ala- Ala- Lys- Arg- Arg- Leu- Pro- pFPhe - NH2 (SEQ ID No: 146)

H- Ala- Ala- Lys- Arg- Arg- Leu- Bug- pFPhe - NH2 (SEQ ID No: 147)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ser- pFPhe - NH2 (SEQ ID No: 148)

H- Ala- Ala- Lys- Arg- Arg- Leu- Asp- pFPhe - NH2 (SEQ ID No: 149)

H- Ala- Ala- Lys- Arg- Arg- Leu- Asn- pFPhe - NH2 (SEQ ID No: 150)

H- Ala- Ala- Lys- Arg- Arg- Leu- pFPhe- Phe - NH2 (SEQ ID N0: 151)

H- Ala- Ala- Lys- Arg- Arg- Leu- diClPhe- Phe - NH2 (SEQ ID No: 152)

H- Ala- Ala- Lys- Arg- Arg- Leu- pClPhe- Phe - NH2 (SEQ ID No: 153)

H- Ala- Ala- Lys- Arg- Arg- Leu- mClPhe- Phe - NH2 (SEQ ID No: 154)

H- Ala- Ala- Lys- Arg- Arg- Leu- oClPhe- Phe - NH2 (SEQ ID No: 155)

H- Ala- Ala- Lys- Arg- Arg- Leu- pIPhe- Phe - NH2 (SEQ ID No: 156)

H- Ala- Ala- Lys- Arg- Arg- Leu- TyrMe- Phe - NH2 (SEQ ID No: 157)

H- Ala- Ala- Lys- Arg- Arg- Leu- Thi- Phe - NH2 (SEQ ID No: 158)

H- Ala- Ala- Lys- Arg- Arg- Leu- Pya- Phe - NH2 (SEQ ID No: 159)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- diClPhe - NH2 (SEQ ID No: 160)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- pClPhe - NH2 (SEQ ID No: 161)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- mClPhe - NH2 (SEQ ID No: 162)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- oClPhe - NH2 (SEQ ID No: 163)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- Phg - NH2 (SEQ ID No: 164)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- TyrMe - NH2 (SEQ ID No: 165)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- Thi - NH2 (SEQ ID No: 166)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- Pya - NH2 (SEQ ID No: 167)

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- Inc - NH2 (SEQ ID No: 168)

and the cyclic peptides:

5,8-cyclo-[H-His-Ala-Lys-Arg-Lys-Leu-Phe-Gly] (SEQ ID No: 169)

5,8-cyclo-[H-His-Ala-Lys-Arg-Orn-Leu-Phe-Gly] (SEQ ID No: 170)

In another aspect, the invention pertains to a peptide of the formula III or IV; $H'X_2K'R_1R_2L'X_5F$ (formula III) (SEQ ID No. 3) or $H'X_2K'R_1R_2L'FX_5$ (formula IV) (SEQ ID No. 189) or a variant thereof, wherein:

H' is nothing, His, D-His, Ala, Thi, Hse, Phe, or Dab;

X2 is Ala, Ser, Abu, Val;

K' is Lys, Arg, or Abu;

R_1 is Arg, Lys, or Gln; and

R_2 is Arg, forms a cyclic peptide with the C-terminal residue, Ser, or Cit;

L' is Leu or Ile;

X_5 is Ile, Leu, Gly, or Ala; and

F' is Phe, para-fluoroPhe, meta-fluoroPhe, L-Psa, 2-Nap,Dhp, or D-Psa.

In one embodiment, X_2 is alanine.

In one embodiment, X_5 isoleucine.

In another embodiment, the invention pertains to a peptide of the formula IV $H'X_2K'R_1R_2L'F'X_5$ (SEQ ID No: 189).

In another embodiment, the peptide is in a cyclic form by virtue of a linkage between the C-terminal residue and the residue 3 upstream to it.

In another embodiment, X_2 is Ala and X_5 is Ile.

In yet another embodiment, F' is para-fluoro-Phe and H' is Ala or nothing.

In another embodiment, K' is Abu; R_1 is Gln; R_2 is Cit or Ser; and X_5 is Ala.

In still another embodiment, a peptide is selected from the group consisting of:

H- his- Ala- Lys- Arg- Arg- Leu- Ile- Phe -NH2 SEQ ID No: 171

H- Ala- Ala- Lys- Arg- Arg- Leu- Ile- Phe -NH2 SEQ ID No: 172

H- Ala- Lys- Arg- Arg- Leu- Ile- Phe -NH2 SEQ ID No: 173

H- Thi- Ala- Lys- Arg- Arg- Leu- Ile- Phe -NH2 SEQ ID No: 174

H- Hse- Ala- Lys- Arg- Arg- Leu- Ile- Phe -NH2 SEQ ID No: 175

H- Phe- Ala- Lys- Arg- Arg- Leu- Ile- Phe -NH2 SEQ ID No: 176

H- Dab- Ala- Lys- Arg- Arg- Leu- Ile- Phe -NH2 SEQ ID No: 177

H-	His-	Abu-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No: 178
H-	His-	Val-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No: 179
H-	His-	Ala-	Arg-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No: 180
H-	His-	Ala-	Lys-	Arg-	Arg-	Ile-	Ile-	Phe	-NH2	SEQ ID No: 181
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Leu-	Phe	-NH2	SEQ ID No: 182
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No: 99
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	2Nap	-NH2	SEQ ID No: 103
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	D-Psa	OH	SEQ ID No: 111
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	Dhp	OH	SEQ ID No: 115
H-	Ala-	Ala-	Abu-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No: 118
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No: 119
H-	Ala-	Ala-	Lys-	Arg-	Cit-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No: 120
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ala-	pFPhe	-NH2	SEQ ID No: 121
H-	Ala-	Ala-	Abu-	Arg-	Ser-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No: 122
H-	Ala-	Ala-	Lys-	Gln-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No: 123
	H-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No: 183

In another aspect, the invention pertains to an assay for identifying candidate substances capable of binding to a cyclin associated with a G1 control CDK enzyme and/or inhibition of said enzyme, comprising; (a) bringing into contact i) a p21 derived peptide as defined in claim 1, ii) said cyclin or portion thereof or cyclin groove, iii) said CDK or portion thereof and iv) said candidate substance, under conditions wherein, in the absence of the candidate substance being an inhibitor of the cyclin/CDK interaction, the p21 derived peptide would bind to said cyclin or portion thereof or cyclin groove, and (b) monitoring any change in the expected binding of the p21 derived peptide and the cyclin or portion thereof or cyclin groove.

In yet another aspect, the invention pertains to an assay for the identification of

compounds that interact with a cyclin or a cyclin when complexed with the physiologically relevant CDK, comprising; (a) incubating a candidate compound and peptide of formula I: $X_1X_2X_3RX_4LX_5F$ (formula II) (SEQ ID No. 2) wherein X_1, X_3, X_4 and X_5 may be any amino acid and X_2 is serine or alanine; and variants thereof or a peptide of the formula III or IV: $H'X'_2K'R_1R_2L'X'_5F'$ (formula III) (SEQ ID No. 3) or $H'X'_2K'R_1R_2L'F'X'_5$ (formula IV) (SEQ ID No: 189) or a variant thereof, wherein H' is His, nothing, D-His, Ala, Thi, Hse, Phe, or Dab; X'_2 is Ala, Ser, Abu, Val; K' is Lys, Arg, or Abu; R_1 is Arg, Lys, or Gln; and R_2 is Arg, forms a cyclic peptide with the C-terminal residue, Ser, or Cit; L' is Leu or Ile; X'_5 is Ile, Leu, Gly, or Ala; F' is Phe, para-fluoroPhe, meta-fluoroPhe, L-Psa, 2-Nap,Dhp, or D-Psa and a cyclin or cyclin/CDK complex; **(b)** detecting binding of either the candidate compound or the peptide of formula II or III with cyclin.

In another aspect, the invention pertains to an assay for candidate compounds that interact with a cyclin by virtue of forming associations with at least two of the amino acids corresponding to the cyclin A amino acids L253, I206 and R211.

In yet another aspect of the invention, the candidate compound additionally forms associations with at least one of the amino acids corresponding to the cyclin A amino acids E223, E224, D284, D283, L253, I206 and R211.

In one embodiment, the candidate additionally forms associations with at least one of the amino acids corresponding to the cyclin A amino acids W217, V219, V221, S408, E411, Y225, I213, L214, G257, R250, Q254, T207 and L214.

In still another aspect, the candidate compound additionally forms associations with at least one of the amino acids corresponding to the cyclin A amino acids G222, Y225, I281, E223, E220, V279, A212, V215, L218, Q406, S408, M210, L253, L218, I239, V256 and M200.

In one embodiment, the cyclin is selected from cyclin A, cyclin E or cyclin D.

In another embodiment, the cyclin is cyclin A.

In one embodiment, the assay comprises use of a three dimensional model of a cyclin and a candidate compound.

In another embodiment, at least one of the assay components is bound to a solid phase.

In still another embodiment, the p21 derived peptide is labeled such as to emit a signal when bound to said cyclin.

In another embodiment, the cyclin is labeled such as to emit a signal when bound to the p21 derived peptide.

In one embodiment, one of the assay components is labeled with a fluorescence emitter and the signal is detected using fluorescence polarization techniques.

In another aspect, the invention pertains to a method of using a cyclin in a drug screening assay comprising: (a)selecting a candidate compound by performing rational drug design with a three-dimensional model of said cyclin, wherein said selecting is performed in conjunction with computer modeling; (b)contacting the candidate compound with the cyclin; and (c)detecting the binding affinity of the candidate compound for the cyclin groove; wherein a potential drug is selected on the basis of its having a greater affinity for the cyclin groove than that of a peptide of formula II: $X_1X_2X_3RX_4LX_5F$ (formula II) (SEQ ID No. 2) wherein X_1, X_3, X_4 and X_5 may be any amino acid and X_2 is serine or alanine; and variants thereof or a peptide of formula III or IV: $H'X'_2K'R_1R_2L'X'_5F'$ (formula III) (SEQ ID No. 3) or $H'X'_2K'R_1R_2L'F'X'_5$ (formula IV) (SEQ ID No. 189) or a variant thereof, wherein H' is His, nothing, D-His, Ala, Thi, Hse, Phe, or Dab; X'_2 is Ala, Ser, Abu, Val; K' is Lys, Arg, or Abu; R_1 is Arg, Lys, or Gln; and R_2 is Arg, forms a cyclic peptide with the C-terminal residue, Ser, or Cit; L' is Leu or Ile; X'_5 is Ile, Leu, Gly, or Ala; F' is Phe, para-fluoroPhe, meta-fluoroPhe, L-Psa, 2-Nap,Dhp, or D-Psa.

and/o G1/S cell cycle arrest; cell cycle-related apoptosis; suppression of E2F transcription factor activity; hypophosphorylation of cellular pRb; and in vitro anti-proliferative effects.

In still another aspect, the invention pertains to use of a peptide in the preparation of a medicament for use in (a) inhibition of CDK2 or (b) in the treatment of proliferative disorders such as cancers and leukaemias where inhibition of CDK2 would be beneficial.

Brief Description of the Drawings

Figure 1 shows the effect of p21 (149-160) on CDK2-Cyclin E induced phopshorylation of different concentrations Histone 1. Yellow line (+ marks)– 1mg/ml Histone1, purple line (diamonds), 0.7 mg/ml Histone 1, blue line (x marks)– 0.25 mg/ml Histone 1 and brown line (closed circles) – 0.1 mg/ml Histone 1.

Figure 2 shows that p21 (141-160)153A is a strong inhibitor of GST-Rb phopshorylation but not of Histone 1 phosphorylation induced by CDK2-Cyclin E kinase complex.

Figure 3 (a) shows interactions of p27(^{27}Ser-Ala-Cys-Arg-Asn-Leu-Phe-Gly^{34}) (SEQ ID No. 190) segment with cyclin A groove (Russo, A. A.; Jeffrey, P. D.; Patten, A. K.; Massague, J.; Pavletich, N. P. *Nature* **1996**, *382*, 325-31). Panel B shows conformation of the same segment (top) compared with modelled cyclic Ser-Ala-Cys-Arg-Lys-Leu-Phe-Gly peptide (SEQ ID No. 191) (bottom).

Figure 4 shows the 3-D structure of the peptide H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No. 28) / cyclin A complex was generated using molecular docking techniques. The peptide structure is represented in black, while only the residues of the cyclin groove that make intermolecular contacts with the peptide are shown. The backbone of cyclin A is represented by the grey ribbon.

Figure 5 shows comparison of the conformation of cyclin A-complexed structures of the p21- and p27-derived peptides H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe (SEQ ID No. 28) and H-Ser-Ala-Cys-Arg-Asn-Leu-Phe-Gly-NH_2 (SEQ ID No. 190). The positioning of the Leu and Phe side chains of the Leu-Ile-

Phe and Leu-Phe-Gly motifs in the groove is remarkably similar, despite the different sequence order of these residues.

Figure 6 shows comparison of modelled cyclin A groove-bound conformations of the p21(152-159)Ser153Ala peptides containing either Phe^{159} (top) or $pFPhe^{159}$ (bottom).

Detailed Description of the Invention

Although the peptides of the first aspect and in some embodiments of the second aspect, include the described CDK4-inhibitory motif RRLIF, the peptides of the present invention have been shown to display preferential selectivity for CDK2 over CDK4 in contrast to those described in Ball et al.(supra) who concluded that such p21 carboxy–terminal peptides "do not have high specific activity for CDK2 inhibition, they are potent inhibitors of CDK4 activity". Thus, Ball et al. do not focus upon this region for further development for preferential CDK2 inhibitors, indeed $p21_{141-160}$ was shown by these authors to be 40 times more active against cyclinD1/CDK4 than cyclinE/CDK2. Thus, further surprising advantages of the above peptides relate to their specificity, particularly for G1 control CDK's, such as CDK2/cyclinE and CDK2/cyclin A, as opposed to mitotic control enzymes including CDK's such as CDK1/cyclin B or A and protein kinase Cα (PKCα).

Further evidence of the unexpected observation that these peptides display activity against CDK4 and CDK2 is that Ball et al. described how N-terminal truncation of $p21_{141-160}$ reduced CDK4/cyclin D1 inhibitory activity. The disclosure therein of RRLIF (SEQ ID No. 184) as being the CDK4-inhibitory motif was made on a theoretical basis rather than a demonstration that a peptide of that size would retain inhibitory activity. Furthermore, of the prior art disclosures discussed above, only two 8-mer peptides have been shown to be active against cyclin A/CDK2, these being the E2F1 derived peptides PVKRRLFG (SEQ ID No. 187) and PVKRRLDL (SEQ ID No. 186). Thus, the present invention has demonstrated, in contrast to the information available in the art, that shorter, in some cases more specific and/or potent inhibitors of cyclin-CDK, especially cyclin E/CDK2 and cyclin A/CDK2 interaction may derived from

within the sequence $p21_{141-160}$.

In one embodiment of the first aspect of the invention, the peptide may include a further amino acid residue at either the N- or C-terminus. The further residue is preferably selected from the polar residues C, N, Q, S, T and Y, and is preferably threonine when added to the N-terminus and serine, when added to the C-terminus. These last recited preferred embodiments correspond to the sequences 148-159 and 149-160 of p21 respectively. In an alternative embodiment, upto 7 amino acid residues may be deleted from the N-terminal end of formula I. Such truncation may therefore give rise to peptides corresponding to p21(150-159), p21(151-159), p21(152-159), p21(153-159),–p21(154-159) p21(155-159) and p21(156-159) or wherein an additional serine residue is added to the C-terminal end to p21(150-160), p21(151-160), p21(152-160), p21(153-160), p21(154-160), p21(155-160) and p21(156-160). Preferably, from 2 to 7 residues are deleted, most preferably seven are deleted. In each of these preferred embodiments it is preferable that, when present the serine residue corresponding to p21(153) is replaced by an alanine residue.

Considering the second aspect of the invention, peptides and variants of the formula $X_1X_2X_3RX_4LX_5F$ (SEQ ID No. 2) include peptides where one or more of:
(a) X1 may be deleted or may be any amino acid,
(b) X2 may be serine or alanine or a straight or branched chain amino,
(c) X3 may be a basic amino acid or straight or branched chain aliphatic amino acid,
(d) R may be unchanged or conservatively substituted (by basic amino acids),
(e) X4 may be any amino acid that is capable of providing at least one site for participating in hydrogen bonding,
(f) L may be unchanged or conservatively substituted,
(g) X5 may be any amino acid, or
(h) F may be unchanged or substituted by any aromatic amino acid.

More particularly, X_2 is preferably alanine as this provides a significant increase in the

L-thioproline*, methyl derivatives of phenylalanine (Phe) such as 4-methyl-Phe*, pentamethyl-Phe*, L-Phe (4-amino)#, L-Tyr (methyl)*, L-Phe (4-isopropyl)*, L-Tic (1;2,3,4-tetrahydroisoquinoline-3-carboxyl acid)*, L-diaminopropionic acid # and L-Phe (4-benzyl)*. The notation * has been utilised for the purpose of the discussion above, to indicate the hydrophobic nature of the derivative whereas # has been utilised to indicate the hydrophilic nature of the derivative, #* indicates amphipathic characteristics. The structures and accepted three letter codes of some of these and other unnatural amino acids are given in the Examples section.

With particular reference to the first aspect of the invention (SEQ ID No. 1), a variant peptide may involve the replacement of an amino acid residue by an alanine residue. In the first aspect of the present invention, such substitution preferably takes place at any of positions 150, 151, 152, 153, 154, 158 or 160 which all display a greater selectivity for CDK2/cyclin E inhibition than CDK4/cyclin D1 inhibition as described below. Most preferably, such alanine replacement occurs at position 153 where in addition to an increase in selectivity, the observed IC_{50} is at least two orders of magnitude greater that for the corresponding parent peptide ($p21_{149-160}$). In respect of the second aspect of the invention, it is also preferable that amino acid replacement is by an alanine residue, most preferably at the 153 position (X_2). Furthermore, in respect of this aspect of the invention, the variant may include the deletion of the N-terminal asparagine residue resulting in the peptide corresponding to p21 (150-159). According the first aspect, a preferable peptide is one including a serine residue at the C-terminus such as the peptide D F Y H A K R R L I F S (SEQ ID No. 10).

As discussed above, variants also include inversion of the two C-terminal amino acid residues and cyclic peptides, both of which are preferred independently as well as when taken together or in combination with any other variant. When such a variant is applied to the second or third aspects of the invention, it is to the exclusion of the peptide PVKRRLFG (SEQ ID No. 187), unless in cyclic form.

With regard to cyclic peptides, these are preferably formed by linkage between the C-terminal amino acid residue and any upstream amino acid residue, preferably 3 amino acid residues upstream to it. Those skilled in the art will be aware as to the nature of such cyclic linkages. In some instances the participating amino acids may require modification in order to facilitate such linkage. In the context of the present invention, cyclic peptides are most conveniently prepared using variants wherein the two C-terminal amino acids are reversed, I and F when considering the first aspect of the invention, X_5 and the terminal phenylalanine residue in the second aspect etc. resulting in a linkage between I or X_5 and an upstream residue. In such circumstances the terminal amino acid residue (I or X_5) is preferably modified to be glycine, the upstream amino acid residue preferably being modified to be lysine or ornithine.

Thus, in accordance with the first aspect of the invention, the peptide may be selected from:

D F Y H A K R R L I F S (SEQ ID No. 10),

T D F Y H S K R R L I F (SEQ ID No. 5),

A F Y H S K R R L I F S (SEQ ID No. 6),

D A Y H S K R R L I F S (SEQ ID No.7),

D F A H S K R R L I F S (SEQ ID No. 8),

D F Y A S K R R L I F S (SEQ ID No. 9),

D F Y H A K R R L I F S (SEQ ID No. 10),

D F Y H S A R R L I F S (SEQ ID No. 11),

D F Y H S K R A L I F S (SEQ ID No. 12),

D F Y H S K R R L A F S (SEQ ID No. 13),

D F Y H S K R R L I F A (SEQ ID No. 14),

F Y H S K R R L I F S (SEQ ID No. 15),

Y H S K R R L I F S (SEQ ID No. 16),

H S K R R L I F S (SEQ ID No. 17),

D F Y H S K R R L I F (SEQ ID No. 18),

F Y H S K R R L I F (SEQ ID No. 19),

Y H S K R R L I F (SEQ ID No. 20),

H S K R R L I F (SEQ ID No. 21),

S K R R L I F (SEQ ID No. 22),

K R R L I F (SEQ ID No. 23),

H-	Arg-	Leu-	Ile-	Phe	$-NH_2$					SEQ ID No. 24
H-	Arg-	Arg-	Leu-	Ile-	Phe	$-NH_2$				SEQ ID No. 25
H-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	$-NH_2$			SEQ ID No. 26
H-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	$-NH_2$		SEQ ID No. 27
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	$-NH_2$	SEQ ID No. 28
H-	Asn-	Leu-	Phe-	Gly	$-NH_2$					SEQ ID No. 29
H-	Arg-	Asn-	Leu-	Phe-	Gly	$-NH_2$				SEQ ID No. 30
H-	Abu-	Arg-	Asn-	Leu-	Phe-	Gly	$-NH_2$			SEQ ID No. 31
H-	Ala-	Abu-	Arg-	Asn-	Leu-	Phe-	Gly	$-NH_2$		SEQ ID No. 32
H-	Ser-	Ala-	Abu-	Arg-	Asn-	Leu-	Phe-	Gly	$-NH_2$	SEQ ID No. 33

Considering $X_1X_2X_3RX_4LX_5F$ (SEQ ID No. 2), preferred peptides and variants thereof may include any one of or optionally at least one or more of the following;

(a) X_1 is histidine, deleted or replaced by a natural or unnatural amino acid residue-such as alanine, 3-pyridylalanine (Pya), 2-thienylalanine (Thi), homoserine (Hse), phenylalanine, or diaminobutyric acid (Dab),

(b) X_2 is alanine or an alternative natural or unnatural amino acid residue having a smaller or slightly larger aromatic or aliphatic side chain, such as glycine, aminobutyric acid (Abu), norvaline (Nva), t-butylglycine(Bug), valine, isoleucine, phenylglycine (Phg) or phenylalanine,

(c) X_3 is lysine or either a basic residue such as arginine or an uncharged natural or unnatural amino acid residue, such as norleucine (Nle), aminobutyric acid (Abu) or leucine,

(d) arginine is replaced by either a basic residue such as lysine or an uncharged natural or unnatural amino acid residue, such as citrulline (Cit), homoserine, histidine, norleucine (Nle) or glutamine,

(e) X_4 is or a natural or unnatural amino acid residue, such as asparagine, proline, serine, aminoisobutyric acid (Aib) or sarcosine (Sar), or an amino acid residue capable of forming a cyclic linkage such as lysine or ornithine,

(f) leucine is replaced with a natural or unnatural amino acid residue having a slightly larger aromatic or aliphatic side chain, such as norleucine, norvaline, cyclohexylalanine (Cha), phenylalanine or 1-naphthylalanine (1Nal),

(g) X_5 is isoleucine or an alternative natural or unnatural amino acid residue having a slightly larger aromatic or aliphatic side chain, such as norleucine, norvaline, cyclohexylalanine (Cha), phenylalanine or 1-naphthylalanine (1Nal),

(h) phenylalanine is replaced with a natural or unnatural amino acid such as leucine, cyclohexylalanine (Cha), homophenylalanine (Hof), tyrosine, para-fluorophenylalanine (pFPhe), meta-fluorophenylalanine (mFPhe), trptophan, 1-naphthylalanine (1Nal), 2-naphthylalanine (2Nal), biphenylalanine(Bip) or (Tic),

(i) X_5 and the terminal phenylalanine residue are reversed, or

(j) the peptide is in cyclic form by for example, the formation of a linkage between the side chain of X_4 and the C-terminus residue.

In accordance with the second embodiment of the invention, the peptide may be selected from;

H S K R R L I F (SEQ ID No. 34),

H A K R R L I F (SEQ ID No. 35),

H S K R R L F G (SEQ ID No. 36),

H A K R R L F G (SEQ ID No. 37),

K A C R R L F G (SEQ ID No. 38),

K A C R R L I F (SEQ ID No. 39).,

	X1	X2	X3	R	X4	L	X5	F		
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 55
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 40
	H-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 41
H-	Pya-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 42
H-	Thi-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 43
H-	Hse-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 44
H-	Phe-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 45
H-	Dab-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 46
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 55

H-	His-	Gly-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 47
H-	His-	Abu-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 48
H-	His-	Nva-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 49
H-	His-	Bug-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 50
H-	His-	Val-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 51
H-	His-	Ile-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 52
H-	His-	Phg-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 53
H-	His-	Phe-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 54
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 55
H-	His-	Ala-	Ala-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 56
H-	His-	Ala-	Nle-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 57
H-	His-	Ala-	Abu-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 58
H-	His-	Ala-	Leu-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 59
H-	His-	Ala-	Arg-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 60
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 55
H-	His-	Ala-	Lys-	Ala-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 61
H-	His-	Ala-	Lys-	Cit-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 62
H-	His-	Ala-	Lys-	Hse-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 63
H-	His-	Ala-	Lys-	His-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 64
H-	His-	Ala-	Lys-	Nle-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 65
H-	His-	Ala-	Lys-	Gln-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 66
H-	His-	Ala-	Lys-	Lys-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 67
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 55
H-	His-	Ala-	Lys-	Arg-	Ala-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 68
H-	His-	Ala-	Lys-	Arg-	Asn-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 69
H-	His-	Ala-	Lys-	Arg-	Pro-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 70
H-	His-	Ala-	Lys-	Arg-	Ser-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 71
H-	His-	Ala-	Lys-	Arg-	Aib-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 72
H-	His-	Ala-	Lys-	Arg-	Sar-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 73
H-	His-	Ala-	Lys-	Arg-	Cit-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 74
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 75
H-	His-	Ala-	Lys-	Arg-	Arg-	Ala-	Ile-	Phe	-NH2	SEQ ID No. 76
H-	His-	Ala-	Lys-	Arg-	Arg-	leu-	Ile-	Phe	-NH2	SEQ ID No. 77
H-	His-	Ala-	Lys-	Arg-	Arg-	Ile-	Ile-	Phe	-NH2	SEQ ID No. 78
H-	His-	Ala-	Lys-	Arg-	Arg-	Val-	Ile-	Phe	-NH2	SEQ ID No. 79
H-	His-	Ala-	Lys-	Arg-	Arg-	Nle-	Ile-	Phe	-NH2	SEQ ID No. 80
H-	His-	Ala-	Lys-	Arg-	Arg-	Nva-	Ile-	Phe	-NH2	SEQ ID No. 81
H-	His-	Ala-	Lys-	Arg-	Arg-	Cha-	Ile-	Phe	-NH2	SEQ ID No. 82
H-	His-	Ala-	Lys-	Arg-	Arg-	Phe-	Ile-	Phe	-NH2	SEQ ID No. 83
H-	His-	Ala-	Lys-	Arg-	Arg-	1Nap-	Ile-	Phe	-NH2	SEQ ID No. 84
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 55

H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ala-	Phe	-NH2	SEQ ID No. 85
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Leu-	Phe	-NH2	SEQ ID No. 86
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Val-	Phe	-NH2	SEQ ID No. 87
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Nle-	Phe	-NH2	SEQ ID No. 88
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Nva-	Phe	-NH2	SEQ ID No. 89
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Cha-	Phe	-NH2	SEQ ID No. 90
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Phe-	Phe	-NH2	SEQ ID No. 91
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	1Nap-	Phe	-NH2	SEQ ID No. 92
	H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Phe	-NH2	SEQ ID No. 93
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 94
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Leu	-NH2	SEQ ID No. 95
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Cha	-NH2	SEQ ID No. 96
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Hof	-NH2	SEQ ID No. 97
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Tyr	-NH2	SEQ ID No. 98
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 99
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	mFPhe	-NH2	SEQ ID No. 100
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Trp	-NH2	SEQ ID No. 101
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	1Nap	-NH2	SEQ ID No. 102
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	2Nap	-NH2	SEQ ID No. 103
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Lys	-NH2	SEQ ID No. 104
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Tic	-NH2	SEQ ID No. 105
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 55
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	L-Pse	OH	SEQ ID No. 106
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	D-Pse	OH	SEQ ID No. 107
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	L-Pse	OH	SEQ ID No. 108
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	D-Pse	OH	SEQ ID No. 109
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	L-Psa	OH	SEQ ID No. 110
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	D-Psa	OH	SEQ ID No. 111
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	L-Psa	OH	SEQ ID No. 112
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	D-Psa	OH	SEQ ID No. 113
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	Dhp	OH	SEQ ID No. 114
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	Dhp	OH	SEQ ID No. 115
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	Pheol		SEQ ID No. 116
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	Pheol		SEQ ID No. 117
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 55
H-	Ala-	Ala-	Abu-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 118
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 119
H-	Ala-	Ala-	Lys-	Arg-	Cit-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 120
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ala-	pFPhe	-NH2	SEQ ID No. 121
H-	Ala-	Ala-	Abu-	Arg-	Ser-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 122
H-	Ala-	Ala-	Lys-	Gln-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 123
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 124
H-	Gly-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 125

H-	Ala-	Ala-	Lys-	hArg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 126
H-	Ala-	Ala-	Lys-	Ser-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 127
H-	Ala-	Ala-	Lys-	Hse-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 128
H-	Ala-	Ala-	Lys-	Arg-	Lys-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 129
H-	Ala-	Ala-	Lys-	Arg-	Orn-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 130
H-	Ala-	Ala-	Lys-	Arg-	Gln-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 131
H-	Ala-	Ala-	Lys-	Arg-	Hse-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 132
H-	Ala-	Ala-	Lys-	Arg-	Thr-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 133
H-	Ala-	Ala-	Lys-	Arg-	Nva-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 134
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Phg-	Ile-	pFPhe	-NH2	SEQ ID No. 135
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Met-	Ile-	pFPhe	-NH2	SEQ ID No. 136
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Ala-	Ile-	pFPhe	-NH2	SEQ ID No. 137
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Hof-	Ile-	pFPhe	-NH2	SEQ ID No. 138
H-	Ala-	Ala-	Lys-	Arg-	Arg-	hLeu-	Ile-	pFPhe	-NH2	SEQ ID No. 139
H-	Ala-	Ala-	Lys-	Arg-	Arg-	aIle-	Ile-	pFPhe	-NH2	SEQ ID No. 140
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Gly-	pFPhe	-NH2	SEQ ID No. 141
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	βAla	pFPhe	-NH2	SEQ ID No. 142
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Phg-	pFPhe	-NH2	SEQ ID No. 143
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Aib-	pFPhe	-NH2	SEQ ID No. 144
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Sar-	pFPhe	-NH2	SEQ ID No. 145
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Pro-	pFPhe	-NH2	SEQ ID No. 146
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Bug-	pFPhe	-NH2	SEQ ID No. 147
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ser-	pFPhe	-NH2	SEQ ID No. 148
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Asp-	pFPhe	-NH2	SEQ ID No. 149
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Asn-	pFPhe	-NH2	SEQ ID No. 150
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	pFPhe-	Phe	-NH2	SEQ ID No. 151
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	diClPhe	Phe	-NH2	SEQ ID No. 152
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	pClPhe-	Phe	-NH2	SEQ ID No. 153
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	mClPhe	Phe	-NH2	SEQ ID No. 154
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	oClPhe-	Phe	-NH2	SEQ ID No. 155
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	pIPhe-	Phe	-NH2	SEQ ID No. 156
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	TyrMe-	Phe	-NH2	SEQ ID No. 157
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Thi-	Phe	-NH2	SEQ ID No. 158
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Pya-	Phe	-NH2	SEQ ID No. 159
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	diClPhe	-NH2	SEQ ID No. 160
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pClPhe	-NH2	SEQ ID No. 161
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	mClPhe	-NH2	SEQ ID No. 162
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	oClPhe	-NH2	SEQ ID No. 163
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phg	-NH2	SEQ ID No. 164
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	TyrMe	-NH2	SEQ ID No. 165
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Thi	-NH2	SEQ ID No. 166

H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Pya	-NH2	SEQ ID No. 167
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Inc	-NH2	SEQ ID No. 168

and the cyclic peptides;

5,8-cyclo-[H-His-Ala-Lys-Arg-Lys-Leu-Phe-Gly] SEQ ID No. 169
5,8-cyclo-[H-His-Ala-Lys-Arg-Orn-Leu-Phe-Gly] SEQ ID No. 170

With particular reference to SEQ ID No. 3, a variant peptide may additionally involve the replacement of an amino acid residue by an alanine residue, the deletion of X_1 or the reversal of X_5 and the terminal phenylalanine residue. These options are also applicable to the peptide SEQ ID No 3 which may therefore, by way of example result in the peptides X_2KRRLX_5F (SEQ ID No. 3) and HX_2KRRLFX_5 (SEQ ID No. 189). Most preferably, the peptide is H A K R R L I F (SEQ ID No. 35). Further variants those discussed below.

More preferably with respect to H X_2 K R R L X_5 F (SEQ ID No. 3) preferred peptides and variants thereof may include any one of or optionally at least one or more of the following;
(a) His is unchanged, deleted or replaced by D-His, Ala, Thi, Hse, Phe, or Dab,
(b) X_2 is Ala unchanged or replaced by Ser, Abu Bug or Val,
(c) Lys is unchanged or replaced by Arg or Abu,
(d) Arg is unchanged or replaced by Lys, Cit, or Gln,
(e) Arg is unchanged or modified to form a cyclic peptide with the C-terminal residue, or replaced by Cit or Ser,
(f) Leu is unchanged or replaced by Ile,
(g) X_5 is Ile unchanged, replaced by Leu or Gly if reversed with Phe,
(h) Phe is unchanged or replaced by para-fluoroPhe, meta-fluoroPhe, L-Psa, 2-Nap or Dhp,
(i) the two C-terminal residue are reversed, or
(j) the peptide is in cyclic form by virtue of a linkage between the C-terminal residue and the residue 3 upstream to it.

Especially preferred are peptides wherein X_2 is Ala and X_5 is Ile, incorporating more than

one of the above variations particularly where Phe is replaced by para-fluoro-Phe and His is replaced by Ala or is deleted. Of such peptides, especially preferred are those that include further modifications where:

(a) Lys is replaced by Abu,

(b) the first Arg residue is replaced by Gln and

(c) the second Arg residue is replaced by Cit or Ser and,

(d) Ile is replaced by Ala.

Thus, preferred peptides in accordance with the preferred sequence H A K R R L I F (SEQ ID NO. 35) include;

His152										
H-	his-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 171
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 172
	H-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 173
H-	Thi-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 174
H-	Hse-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 175
H-	Phe-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 176
H-	Dab-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 177
Ala153										
H-	His-	Abu-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 178
H-	His-	Val-	Lys-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 179
Lys154										
H-	His-	Ala-	Arg-	Arg-	Arg-	Leu-	Ile-	Phe	-NH2	SEQ ID No. 180
Leu157										
H-	His-	Ala-	Lys-	Arg-	Arg-	Ile-	Ile-	Phe	-NH2	SEQ ID No. 181
Ile158										
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Leu-	Phe	-NH2	SEQ ID No. 182
Phe159										
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 99
H-	His-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	2Nap	-NH2	SEQ ID No. 103
H-	His	Ala	Lys	Arg	Arg	Leu	Ile	D-Psa	OH	SEQ ID No. 111
H-	His	Ser	Lys	Arg	Arg	Leu	Ile	Dhp	OH	SEQ ID No. 115
Multiples										
H-	Ala-	Ala-	Abu-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 118
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 119
H-	Ala-	Ala-	Lys-	Arg-	Cit-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 120
H-	Ala-	Ala-	Lys-	Arg-	Arg-	Leu-	Ala-	pFPhe	-NH2	SEQ ID No. 121
H-	Ala-	Ala-	Abu-	Arg-	Ser-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 122

H-	Ala-	Ala-	Lys-	Gln-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 123
	H-	Ala-	Lys-	Arg-	Arg-	Leu-	Ile-	pFPhe	-NH2	SEQ ID No. 183

The three letter notations appearing above are in accordance with IUPAC convention. The structure of various unnatural amino acid derivatives are provided in the introduction to the Examples, further expansion on nomenclature being given above.

The peptides of the present invention may be subjected to a further modification that is beneficial in the context of the present invention being conversion of the free carboxyl group of the carboxy terminal amino acid residue, to a carboxamide group. By way of example, when the peptide is of SEQ ID No.1 the carboxy terminal phenylalanine residue may have its carboxyl group converted into a carboxamide group. This modification is believed to enhance the stability of the peptide. Thus, the C-terminal amino acid residue may be in the form -C(O)-NRR', wherein R and R' are each independently selected from hydrogen, C1-6 alkyl, C1-6 alkylene or C1-6 alkynyl (collectively referred to "alk"), aryl such as benzyl or alkaryl, each optionally substituted by heteroatoms such as O, S or N. Preferably at least one of R or R' is hydrogen, most preferably, they are both hydrogen. Thus, the present invention therefore encompasses the peptides wherein the C-terminal amino acid residue is in the carboxyl or carboxamide form.

The present invention further encompasses the above described peptides of the first, second and third aspects, their use in the inhibition of CDK2, their use in the treatment of proliferative disorders such as cancers and leukaemias where inhibition of CDK2 would be beneficial and their use in the preparation of medicaments for such use. Such preparation including their use in assays for further candidate compound as described herein. The embodiments described as being preferred in the context of the peptides of the invention apply equally to their use.

ASSAYS

A further embodiment of the present invention relates to assays for candidate substances that are capable of modifying the cyclin interaction with CDK's, especially CDK2 and

Thus the present invention further relates to an assay for the identification of compounds that interact with cyclin A, cyclin E or cyclin D (hereinafter "a cyclin") or these cyclins when complexed with the physiologically relevant CDK, comprising;

(a) incubating a candidate compound and a peptide of the formula $X_1X_2X_3RX_4LX_5F$ (SEQ ID No. 2) or more preferably of formula HX_2KRRLX_5F (SEQ ID No.3) or variants thereof as defined above, and a cyclin or cyclin/CDK complex,

(b) detecting binding of either the candidate compound or the peptide of formula $X_1X_2X_3RX_4LX_5F$ (SEQ ID No. 3)/ HX_2KRRLX_5F (SEQ ID No. 189) with the cyclin.

The assays of the present invention (discussed hereinafter with reference to cyclin A) encompass screening for candidate compounds that bind a cyclin "recruitment center" or "cyclin groove" discussed above in respect of the prior art but herein defined in greater detail with reference to the amino acid sequence of preferably human cyclin A or of partially homologous and functionally equivalent **mammalian** cyclins. The substrate recruitment site from previously described cyclin A/peptide complexes consists mainly of residues of the α1 (particularly residues 207-225) and α3 (particularly residues 250-269) helices, which form a shallow groove on the surface, comprised predominantly of hydrophobic residues. This is discussed in greater detail in Russo AA et al. (Nature (1996) 382, 325-331) with respect to p27/cyclin A. From the X-ray structure assigned to the p27/cyclin A/CDK2 provided therein it is possible to conclude that the sequence SACRNLFG (SEQ ID No. 190) of p27 that interacts with cyclin A does so through the following interaction residues of cyclin A:

p27 residue	Cyclin A residues
S	E220, E224
A	W217, E220, V221, E224, I281
C	Y280, I281, D283
R	D216, W217, E220, Q254

embodiments in competitive binding assays with candidate compounds, further compounds that interact at this site may be identified and assigned utility in the control of the cell cycle by virtue of controlling, preferably inhibiting CDK2 and/or CDK4 activity. Such assays may be performed in vitro or virtually i.e. by using a three dimensional model or preferably, a computer generated model of a complex of a peptide of the present invention and cyclin A. Using such a model, candidate compounds may be designed based upon the specific interactions between the peptides of the present invention and cyclin A, the relevant bond angles and orientation between those components of the peptides of the present invention that interact both directly and indirectly with the cyclin groove. By way of example, Figure 4 shows the interaction between the peptide HAKRRLIF (SEQ ID No. 35) and Cyclin A. From using the three dimensional model computer generated by this interaction it has been possible to identify the cyclin A amino acid residues that interact with the peptides of the present invention, particularly with HAKRRLIF (SEQ ID No. 35) as outlined above and discussed in greater detail in the examples.

In an embodiment, the cyclin groove includes about residues 173-432 of human cyclin A. In another embodiment, the cyclin groove includes about residues 199-306 of human cyclin A. In a preferred embodiment, the cyclin groove includes about residues 207-225 and about residues 250-269 and about residues 274-282 of human cyclin A. In another embodiment, the cyclin groove includes one or more of: about residues 207-225; about residues 250-269; and about residues 274-282 of human cyclin A. In another embodiment, the cyclin groove includes two or more of: about residues 207-225; about residues 250-269; and about residues 274-282 of human cyclin A

As used herein the term "three dimensional model" includes both crystal structures as determined by X-ray diffraction analysis, solution structures determined by nuclear magnetic resonance spectroscopy as well as computer generated models. Such computer generated models may be created on the basis of a physically determined structure of a peptide of the present invention bound to cyclin A or on the basis of the known crystal structure of cyclin A, modified (by the constraints provided by the software) to

accommodate a peptide of formula I. Suitable software suitable of the generation of such computer generated threedimensional models include AFFINITY, CATALYST and LUDI (Molecular Simulations, Inc.).

Such three dimensional models may be used in a program of rational drug design to generate further candidate compounds that will bind to cyclin A. As used herein the term "rational drug design" is used to signify the process wherein structural information about a ligand-receptor interaction is used to design and propose modified ligand candidate compounds possessing improved fit with the receptor site in terms of geometry and chemical complementarity and hence improved biological and pharmaceutical properties, such properties including, *e.g.*, increased receptor affinity (potency) and simplified chemical structure. Such candidate compounds may be further compounds or synthetic organic molecules. The preferred peptides for use in these aspects of the invention are identical to those designated as preferred with respect to the first and second aspects of the invention, most especially those of the formula HX_2KRRLX_5F (SEQ ID No. 3) and of those particularly the peptide HAKRRLIF (SEQ ID No. 35). In a preferred embodiment, rational drug design is focussed upon the four C-terminal amino acids RLX_5F or $RLFX_5$ or variants thereof as discussed above with respect to SEQ ID No. 3.

Using techniques known in the art, crystal or solution structures of cyclin A bound to a peptide of the present invention may be generated, these too may be used in a **programme** of rational drug design as discussed above.

Crystals of the p21 derived peptides of the present invention complexed with cyclin A can be grown by a number of techniques including batch crystallization, vapor diffusion (either by sitting drop or hanging drop) and by microdialysis. Seeding of the crystals in some instances is required to obtain X-ray quality crystals. Standard micro and/or macro seeding of crystals may therefore be used.

Once a crystal of the present invention is grown, X-ray diffraction data can be collected.

Crystals can be characterized by using X-rays produced in a conventional source (such as a sealed tube or a rotating anode) or using a synchrotron source. Methods of characterization include, but are not limited to, precision photography, oscillation photography, diffractometer data collection, and Se-Met multiwavelength anamalous dispersion data.

Once the three-dimensional structure of a protein-ligand complex formed between a p21 derived peptide of the present invention and cyclin A is determined, a candidate compound may be examined through the use of computer modeling using a docking program such as GRAM, DOCK or AUTODOCK [Dunbrack et al., 1997, Folding & Design 2:R27-42]. This procedure can include computer fitting of candidate compounds to the ligand binding site to ascertain how well the shape and the chemical structure of the candidate compound will complement the binding site. [Bugg et al., *Scientific American*, December:92-98 (1993); West et al;l *TIPS*, 16:67-74 (1995)]. Computer programs can also be employed to estimate the attraction, repulsion and steric hindrance of the two binding partners (i.e. the ligand-binding site and the candidate compound). Generally the tighter the fit, the lower the steric hindrances, and the greater the attractive forces, the more potent the potential drug since these properties are consistent with a tighter binding constant. Furthermore, the more specificity in the design of a potential drug the more likely that the drug will not interact as well with other proteins. This will minimize potential side-effects due to unwanted interactions with other proteins.

Initially candidate compounds can be selected for their structural similarity to a p21 derived peptide of the present invention such as HAKRRLIF (SEQ ID No. 35), the four C-terminal amino acids thereof RLX_5F or $RLFX_5$; or variants or a region thereof. The structural analog can then be systematically modified by computer modeling programs or by inspection until one or more promising candidate compounds are identified. A candidate compound could be obtained by initially screening a random peptide library produced by recombinant bacteriophage for example [Scott and Smith, *Science*, 249:386-390 (1990); Cwirla et al., *Proc. Natl. Acad. Sci.*, 87:6378-6382 (1990); Devlin et al., *Science*, 249:404-406 (1990)].

sorting, FACS; or TUNEL assay), suppression of E2F transcription factor activity (e.g. using a cellular E2F reporter gene assay), hypophosphorylation of cellular pRb (using Western blot analysis of cell lysates with relevant phospho-specific antibodies), or generally in vitro anti-proliferative effects.

Thus, a further related aspect of the present invention relates to a three dimensional model of a peptide of the formula $X_1X_2X_3RX_4LX_5F$ (SEQ ID No. 2) or preferably HX_2KRRLX_5F (SEQ ID No. 3): or variants thereof as defined above and cyclin A.

The invention further includes a method of using a three-dimensional model of cyclin A and a peptide of the present invention in a drug screening assay comprising;
(a) selecting a candidate compound by performing rational drug design with the three-dimensional model, wherein said selecting is performed in conjunction with computer modeling;
(b) contacting said candidate compound with cyclin A, and
(c) detecting the binding of the candidate compound; wherein a potential drug is selected on the basis of the candidate compound having a similar or greater affinity for cyclin A than that of a standard p21 derived peptide.

In a preferred embodiment the standard p21 derived peptide has the general formula HX_2KRRLX_5F (SEQ ID No. 3) as defined above. Preferably, the three dimensional model is a computer generated model.

The peptides of the invention and substances identified or identifiable by the assay methods of the invention may preferably be combined with various components to produce compositions of the invention. Preferably the compositions are combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition (which may be for human or animal use). Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition of the invention may be administered by direct injection. The composition may be formulated for

sampling using molecular dynamics calculated over 5 ps in 100 fs stages, where the temperature is scaled from 500 K to 300 K. The calculation was completed by a final **minimization** over 1,000 steps using the Polak-Ribiere Conjugate Gradient method.

Example 5: Structure-activity relationships of p21(145-164) peptides with respect to inhibition of cyclin E/CDK2 and cyclin D1/CDK4

Previous studies have shown that a 20-residue peptide, derived from the *C*-terminus of $p21^{WAF1}$ (residues 141-160) binds to CDK4 and cyclin D1 and is able to inhibit *in vitro* kinase activity of the CDK4/cyclin D1 complex (Ball, K. L.; Lain, S.; Fåhraeus, R.; Smythe, C.; Lane, D. P. *Curr. Biol.* 1996, *7*, 71-80). In order to define the pharmacophore region of the $p21^{WAF1}$ *C*-terminus, 12mer overlapping peptides covering the region of p21(145-164) were synthesized. The *in vitro* effect of these peptides on CDK4/cyclin D1 and CDK2/cyclin E kinase activity in terms of inhibition of phosphorylation of GST-pRb was investigated.

A shorter sequence being a 12 amino acid peptide DFYHSKRRLIFS – p21 (149-160) (SEQ ID No. 1) was found to have very similar activity as the original 20-mer peptide of Ball et al. with respect to in vitro inhibitory activity in vitro CDK4-Cyclin D1 kinase.

A detailed SAR analysis of p21 (149-160) was done in 96-well format CDK4-Cyclin D1 kinase assay using different peptide derivatives – truncations and alanine substitutions. In order to determine the relative importance of each position of the 12 amino acid peptide which contained the binding domain, p21 (149-160) derivatives were synthesized in which each residue was sequentially substituted with Ala. The effect of the peptide mutations on their kinase inhibitory activity was then tested. Ala substitution of Phe^{150}, Tyr^{151}, His^{152}, Ile^{158}, and Ser^{160} did not change significantly the CDK2/cyclin E inhibitory activity of p21(149-160). Substitution of Ser^{153} with Ala increased 100-fold the inhibitory potency of p21(149-160) towards CDK2/cyclin E. The results are shown in Table 1.

SAR of p21 (149-160) in CDK2/Cyclin E kinase assay.

Table 1: Structure-activity relationships of p21(145-164) peptides with respect to inhibition of cyclin E/CDK2 and cyclin D1/CDK4

P21[wild] Sequence[a]	Sequence ID No.	Formula	M_r	MS[b]	RP-HPLC[c]		Kinase Inhibition[d]			
							Cyclin E/CDK2		Cyclin D1/CDK4	
145 150 155 160 164				[M+H]	T_r (min)	Purity (%)	IC_{30}(μM)	% Inhibition	IC_{50}(μM)	% Inhibition
S M T D F Y H S K R R L I F S K R K P	193									
S M T D F Y H S K R R	194	$C_{65}H_{102}N_{22}O_{19}S$	1527 71	1530.3	11.0	55.7	-	35	-	30
S M T D F Y H S K R R L	195	$C_{67}H_{106}N_{22}O_{18}S$	1539 76	1541.66	11.5	73.5	-	40	-	18
M T D F Y H S K R R L I	196	$C_{70}H_{112}N_{22}O_{17}S$	1565.84	1569.5	12.2	93.5	-	35	-	11
T D F Y H S K R R L I F	5	$C_{74}H_{112}N_{22}O_{17}$	1581.82	1583.9	13.3	76.9	2.2 ± 0 4	85	15 ± 3	72
D F Y H S K R R L I F S	4	$C_{73}H_{110}N_{2}20_{17}$	1567 79	1569 7	12 8	92 7	4.5 ± 0 5	80	20 ± 2	70
F Y H S K R R L I F S K	197	$C_{75}H_{117}N_{23}O_{15}$	1580.88	1580.4	12 0	89 4	26 ± 6 2	70	41 ± 10	70
Y H S K R R L I F S K R	198	$C_{72}H_{120}N_{26}O_{15}$	1589 89	1592 0	11 2	90 3	17 6 ± 6.9	80	45 ± 10	60
H S K R R L I F S K R K	199	$C_{69}H_{123}N_{27}O_{14}$	1554 89	1556.9	10.7	20.0	8 7 ± 2.5	90	34 ± 6	80
S K R R L I F S K R K P	200	$C_{68}H_{123}N_{25}O_{14}$	1514 86	1518 7	10 7	87 9	46 ± 33	70	-	40
A F Y H S K R R L I F S	6	$C_{72}H_{110}N_{22}O_{15}$	1523 78	1526.0	12.7	92.3	11 ± 2	70	22 ± 4	72
D *A* Y H S K R R L I F S	7	$C_{67}H_{106}N_{22}O_{17}$	1491.70	1494.8	12.2	80.8	5.9 ± 0.4	85	37 ± 6	76
D F *A* H S K R R L I F S	8	$C_{67}H_{106}N_{22}O_{16}$	1475.70	1482.2	12.5	91.2	5.3 ± 0.6	80	121 ± 31	56
D F Y *A* S K R R L I F S	9	$C_{70}H_{108}N_{20}O_{17}$	1501 73	1506.6	13.0	79.1	5.1 ± 0 .5	80	73 ± 42	60
D F Y H *A* K R R L I F S	10	$C_{73}H_{110}N_{22}O_{16}$	1551.79	1554.2	12.9	97.8	0.04 ± 0.005	80	10	52
D F Y H S *A* R R L I F S	11	$C_{70}H_{103}N_{21}O_{17}$	1510.7	1512.9	13.8	91 6	12.9 ± 2.4	80	200	50
D F Y H S K *A* R L I F S	201	$C_{70}H_{103}N_{19}O_{17}$	1482.68	1485 7	13.3	72.9	-	25	-	30
D F Y H S K R *A* L I F S	12	$C_{70}H_{103}N_{19}O_{18}$	1483 68	1488 9	13.2	78.6	30 ± 8	70	-	30
D F Y H S K R R *A* I F S	202	$C_{70}H_{104}N_{22}0_{17}$	1525 71	1529.0	12 1	94.5	-	30	-	30
D F Y H S K R R L *A* F S	13	$C_{70}H_{104}N_{22}O_{18}$	1526 71	1527 9	12 0	94.8	14 ± 3	80	53 ± 20	61
D F Y H S K R R L I *A* S	203	$C_{67}H_{106}N_{22}O_{17}$	1491 70	1495 0	11 3	89.6	-	20	-	35
D F Y H S K R R L I F *A*	14	$C_{73}H_{110}N_{22}O_{16}$	1551 79	1551 1	13.1	93 0	5.4 ± 1 1	70	40	60
F Y H S K R R L I F S	15	$C_{69}H_{105}N_{21}O_{14}$	1452 71	1450 2	12 6	83.2	6.8 ± 1.0	80	22 ± 5	70
Y H S K R R L I F S	16	$C_{60}H_{96}N_{20}O_{13}$	1305 53	1304.0	12.2	81 8	7.3 ± 0.8	80	20 ± 1	70
H S K R R L I F S	17	$C_{51}H_{87}N_{19}O_{11}$	1142 36	1141.0	12.0	94 4	3.4 ± 0 2	80	32 ± 6	65
D F Y H S K R R L I F	18	$C_{70}H_{105}N_{21}O_{15}$	1480.72	1476.5	13.5	94.6	2 ± 0.2	75	13 ± 2	70
D F Y H S K R R L I	204	$C_{61}H_{96}N_{20}O_{14}$	1333.54	1331.2	12 1	89.0	-	35	-	20
D F Y H S K R R L	205	$C_{55}H_{85}N_{19}O_{13}$	1220.38	1219.6	10.6	98.0	200	50	-	10
D F Y H S K R R	206	$C_{49}H_{74}N_{18}O_{12}$	1107 23	1106 9	9 8	96.4	-	40	-	10
D F Y H S K R	207	$C_{41}H_{62}N_{14}O_{11}$	951 04	950 8	9.6	89.8	200	50	-	-
D F Y H S K	208	$C_{17}H_{50}N_{10}O_{10}$	794.85	794.4	9.5	96.9	200	45	-	10
F Y H S K R R L I F	19	$C_{66}H_{100}N_{20}O_{12}$	1365 63	1362 6	13 3	85.5	5 8 ± 1	80	19 ± 3	70
F Y H S K R R L I	209	$C_{57}H_{91}N_{19}O_{11}$	1218 45	1218 2	9 6	68 2	-	45	-	20
F Y H S K R R L	210	$C_{51}H_{80}N_{18}O_{10}$	1105.3	1104.5	10 4	86.9	>200	48	-	20
F Y H S K R R	211	$C_{45}H_{69}N_{18}O_{10}$	992 14	994.3	9.2	83.6	>200	45	-	20
F Y H S K R	212	$C_{39}H_{57}N_{13}O_{8}$	835 95	838 2	8 9	92.4	-	20	-	10
Y H S K R R L I F	20	$C_{57}H_{91}N_{19}O_{11}$	1218 45	1218 8	12.9	94.3	7 ± 2	80	16 ± 1	70
Y H S K R R L I	213	$C_{48}H_{82}N_{18}O_{10}$	1071 28	1072 4	10.9	82.5	>200	45	-	15
Y H S K R R L	214	$C_{42}H_{71}N_{17}O_{9}$	958 12	960.4	9.2	95.8	>200	30	-	10
Y H S K R R	215	$C_{36}H_{60}N_{16}O_{8}$	844.96	847 4	7.4	87.2	-	40	-	10
Y H S K R	216	$C_{30}H_{48}N_{12}O_{7}$	688 78	691.2	7.0	66.9	-	25	-	10
H S K R R L I F	21	$C_{48}H_{82}N_{18}O_{9}$	1055.28	1056.5	12.7	81.8	3.4 ± 1	80	21 ± 4	72
S K R R L I F	22	$C_{42}H_{75}N_{15}O_{8}$	918 14	919.3	8.2	93.4	7.7 ± 0.5	80	54	72
K R R L I F	23	$C_{39}H_{70}N_{14}O_{6}$	831 06	823 2	7.4	99.1	11 ± 1.3	80	>200	72
H S K R R L I	273	$C_{39}H_{73}N_{17}O_{8}$	908 11	909 0	10.6	86.7	-	35	-	10
H S K R R L	217	$C_{33}H_{62}N_{16}O_{7}$	794 95	797.5	8 7	89.9	>200	45	-	-
K R R L I F S K	185	$C_{48}H_{87}N_{17}O_{9}$	1046.31	1047 9	11.3	94.2	>200	60	-	20

All peptides were synthesised with free amino termini and as the C-terminal carboxamides

DE MALDI-TOF MS, positive mode, α-cyano-4-hydroxycinnamic acid matrix, calibration using authentic peptides in the appropriate *m/z* range

Vydac218TP54, 1 mL/min, 25 oC, 0 –60 % MeCN in 0.1 % aq CF_3 COOH over 20 min, purity by integration at λ = 214 nm

Standard kinase assay procedures [ATP] = 100 μM

instead of Arg on the 5th position), Ser and LFG, and Ser and LIF containing peptides. The least potent were LDL containing peptides.

These results manifest the importance of Ala and LIF motif for the kinase inhibitory potency of the peptides.

Competitive binding of peptides, containing different motifs (LIF, LFG, LDL) to Cyclin A or Cyclin D1.

The next important question was if these peptides share the same kinase inhibitory mechanism (bind to the same Cyclin docking site). To answer this question we developed a competitive binding assay where the influence of the 8-mers on Cyclin A (D1) – p21 (149-159) binding was studied (See Materials and Methods for more details).

The results from Cyclin D1 competitive binding assay are summarized on Table 6. For easy comparison, the data for CDK4/Cyclin D1 kinase inhibitory activity of the peptides are given in the same table.

Table 5: Kinase inhibitory activity of LDL, LIF and LFG containing peptides, derived from E2F, p107, p21 N- and C-terminus and p27

Peptide	Sequence[a]	SEQ ID No.	Kinase Inhibition								Competitive binding [b]	
			Cyclin A/CDK2		Cyclin E/CDK2		Cyclin D1/CDK4		Cyclin D1/CDK6		Cyclin A	Cyclin D1
			IC_{50} (μM)	% Inhibition	IC_{50} (μM)	% Inhibition	IC_{50} (μM)	% Inhibition	IC_{50} (μM)	% Inhibition	IC_{50} (μM)	IC_{50} (μM)
P21 *C* –terminus	HSKRRLIF	21	3.4	80	3.4	80	21	72	n/d	n/d	N/d	48
P21 *C* –terminus (S153A)	HAKRRLIF	28	0.021	88	0.35	81	6	82	5.8	100	0.3	13
P21 *C* –terminus – LFG hybrid	HSKRRLFG	36	1.4	78	1.6	82	n/a	42	n/d	n/d	4.4	>200
P21 *C* –terminus – LDL hybrid	HSKRRLDL	220	5.4	78	39	74	n/a	24	n/d	n/d	5.8	>200
P21 *C* –terminus (S153A) LFG	HAKRRLFG	37	0.67	78	0.9	82	30	70	n/d	n/d	0.35	33
E2F1	PVKRRLDL	186	1.2	80	2.1	74	99	58	n/d	n/d	1.2	>100
P27	SAURNLFG	33	6.1	80	2	82	n/a	46	n/d	n/d	3.8	>200
P107	SAKRRLFG	222	0.73	75	0.5	86	17	78	n/d	n/d	0.51	24
P21 *N* –terminus	KAURRLFG	268	0.54	80	0.074	86	42	66	n/d	n/d	0.75	134
P21 *N* – terminus – LIF hybrid	KAURRLIF	269	0.062	70	1.2	78	13	83	n/d	n/d	0.3	20

[a] All peptides were synthesised with free amino termini and as the C –terminal carboxamides
[b] Using the immobilised p21 (149-159) peptide biotinyl-Ahx-Asp-Phe-Tyr-His-Ser-Lys-Arg-Arg-Leu-Ile-Phe-NH (Seq. I.D. No. 271)

Table 6: Competitive cyclin A binding of p21 – and pRb(866-877)/ pRb (870-877) peptides

Compound	Sequence ID No.	Formula	M_r	MS[a] [M+H]$^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Competitive cyclin A binding IC_{50} (μM) immobilised pRb peptide[c]	Competitive cyclin A binding IC_{50} (μM) immobilised p21 peptide[d]
H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	28						0.2	0.02
H- Asp- Phe- Tyr- His- Ala- Lys- Arg-Arg-Leu- Ile- Phe - NH_2	223	$C_{70}H_{105}N_{21}O_{14}$	1464.7	1466.0	15.8[i]	>95	0.1	n/d
H- Ser- Asn- Pro- Pro- Lys- Pro- Leu- Lys- Lys- Leu-Arg-Phe-Asp-Ile- Glu - NH_2	224	$C_{82}H_{137}N_{23}O_{21}$	1781.1	1780.0	18.1[ii]	>95	35	48
H- Lys- Pro- Leu- Lys- Lys- Leu-Arg- Phe - NH_2	225	$C_{50}H_{89}N_{15}O_8$	1028.3	1026.0	17.0[iii]	>95	0.6	24
GST-pRb(772-928)[e]	-						n/d	9

[a] DE MALDI-TOF MS, +ve mode, $_{\alpha}$-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)
[b] Vydac 218TP54, 1 mL/min, 25 °C, λ = 214 nm; [i] 0 -40 %[ii] 10.5 – 20.5% MeCN in 0.1 % aq CF_3COOH over 20 min,
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 μM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-Lys-Pro-Leu-Lys-Lys-Leu-Arg--Phe-NH_2 (Seq. ID No. 226)
[e] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Example 11: Competitive binding of p21^{WAF1} and H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No. 28) to cyclin A in the presence and absence of CDK2

p21^{WAF1} contains two cyclin-binding sites, one each in its *N*- and *C*-terminus [(p21(19-23) and p21(154-159)], as well as a CDK2-binding site [p21(46-65)]. The cyclin A-binding affinities of full-length p21^{WAF1} and the peptide containing only the *C*-terminal cyclin-binding motif were compared in the presence and absence of CDK2. This showed (Table 7) that recombinant p21^{WAF1} had *ca.* 27-fold lower affinity than H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No. 28) for cyclin A alone. When cyclin A was pre-complexed with CDK2, on the other hand, the apparent binding affinity of p21^{WAF1} increased and was comparable to that of the octapeptide. The increased ability of p21^{WAF1} to compete with the octapeptide for binding to CDK2-complexed cyclin A is most probably due to the contribution of the CDK-binding motif present in the former. On the other hand, the presence of CDK2 slightly decreased the apparent binding affinity of H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No. 28) for cyclin A, which could be due to some conformational changes of the substrate recognition site on the cyclin sub-unit upon binding of CDK2.

Table 7: Competitive binding of $p21^{WAF1}$ and H-His-Ala-Lys-Arg-Art-Leu-Ile-Phe-NH_2 to cyclin A in presence and absence of CDK2

Protein	Test ligand in solution	Sequence ID No.	Competitive binding IC_{50}(nM)*
Cyclin A	H- His-Ala- Lys-Arg-Arg-Leu- Ile- Phe $-NH_2$	28	14
Cyclin A	human recombinant $p21^{waf1}$		289
Cyclin A / CDK2 complex	H- His-Ala- Lys-Arg-Arg-Leu- Ile- Phe $-NH_2$	28	28
Cyclin A / CDK2 complex	human recombinant $p21^{waf1}$		11

* Competitive binding of cyclin A or cyclin A / CDK2 complex using immobilized peptide biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Examples 12-22: Structure-activity relationships of the p21(152-159)Ser153Ala peptide (H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2) (SEQ ID No. 28)

For the purposes of the following examples, the reference peptide of the invention has been taken as HAKRRLIF (SEQ ID NO. 35) i.e. a preferred peptide of the invention in accordance with the third aspect. As such, the relative activity is expressed against this peptide and all relative activities approaching (over about 0.7) or greater than unity indicate peptides that may be classified as preferred. The comments provided in these Examples are made with this comparator in mind. It should however be borne in mind that even a peptide having a relative activity of <0.1, remains within the scope of the present invention by virtue of still being active in the context of the invention, such variants are variants upon the first or second embodiments as described above.

Example 12: Sensitivity to chiral changes

Each residue in turn was substituted by its chiral antipode and the resulting peptide analogues were tested for both CDK2/cyclin A kinase inhibition and competitive cyclin A binding in the presence of immobilised p21(152-159)Ser153Ala peptide. It was found that inversion of configuration at the $C^{\square}$ atoms was only tolerated (in terms of retention of biological activity) at the peptide's termini. Thus His^{152} could be present as either the L- or D-amino acid without loss of potency. Some potency was lost for the corresponding change at position Ala^{153}. Lys^{154}-Ile^{158} could not be substituted by the corresponding D-amino acids without near-complete loss of activity. Some activity was retained when

Phe[159] was inverted. These results confirm the highly selective and specific binding mode of the lead peptide. The effects seen for the terminal residues probably reflect the fact that these residues are conformationally more flexible in solution than sequence-internal groups and can be brought into a productive binding mode upon binding

Example 12: D-Amino acid substitutions based on p21(152-159)Ser 153Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity Kinase Inhibition[c]	Relative Activity Cyclin A Binding[d]
H- **His- Ala- Lys-Arg- Arg-Leu- Ile- Phe** -NH_2	284	$C_{48}H_{82}N_{18}O_8$					1	1
H- **his**- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	171	$C_{48}H_{82}N_{18}O_8$	1039.3	1039.1	15.4	96	1.7	0.9
H- His- **Ala**- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	224	$C_{48}H_{82}N_{18}O_8$	1039.3	1042.5	15.3	98	0.3	0.6
H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	225	$C_{48}H_{82}N_{18}O_8$	1039.3	1042.9	15.6	100	<0.1	<0.1
H- His- Ala- Lys-**Arg**- Arg-Leu- Ile- Phe -NH_2	229	$C_{48}H_{82}N_{18}O_8$	1039.3	1041.6	15.2	99	<0.1	<0.1
H- His- Ala- Lys-Arg- **Arg**-Leu- Ile- Phe -NH_2	30	$C_{48}H_{82}N_{18}O_8$	1039.3	1041.1	15.2	99	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg-**Leu**- Ile- Phe -NH_2	77	$C_{48}H_{82}N_{18}O_8$	1039.3	1041.0	17.6	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg-Leu- **Ile**- Phe -NH_2	231	$C_{48}H_{82}N_{18}O_8$	1039.3	1040.5	18.1	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg-Leu- Ile- **Phe** -NH_2	233	$C_{48}H_{82}N_{18}O_8$	1039.3	1039.7	17.1	100	0.1	0.2

[a] DE MALDI-TOF MS, +ve mode, $_{\alpha}$-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 28)
[b] Vydac218TP54, 1 mL/min, 25°C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 µM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Residue substitutions

Example 13: His[152]

This residue is comparatively insensitive to substitution. With the exception of Pya, all residue substitutions were either tolerated or even lead to enhanced binding and/or kinase inhibition potency. Furthermore, this residue can be truncated without significant loss in biological activity.

Example 13: Substitutions of His^{152} residue in p21(152-159)Ser153Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity: Kinase Inhibition[c]	Relative Activity: Cyclin A Binding[d]
H- **His**- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- ***Ala***- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	40	$C_{45}H_{80}N_{16}O_8$	973.2	975.4	15.4	98	1.8	2.5
H- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	41	$C_{42}H_{75}N_{15}O_7$	902.1	901.0	15.5	100	1	0.3
H- ***Pya***- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	42	$C_{51}H_{84}N_{16}O_8$	1049.3	1050.6	15.4	98	<0.1	0.2
H- ***Thi***- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	43	$C_{49}H_{82}N_{16}O_8S$	1055.3	1055.5	16.3	100	2	0.4
H- ***Hse***- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	44	$C_{46}H_{82}N_{16}O_9$	1003.3	1002.9	15.7	82	2	2
H- ***Phe***- Ala- Lys-Arg- Arg- Leu- Ile- Phe -NH_2	45	$C_{51}H_{84}N_{16}O_8$	1049.3	1052.3	16.3	100	3	1
H- ***ab***- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	46	$C_{46}H_{83}N_{17}O_8$	1002.3	1004.7	15.5	100	5	0.4

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2,(SEQ ID No: 28)
[b] Vydac218TP54, 1 mL/min, 25 °C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 µM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No: 192)

Example 14: Ala^{153}

This is the residue position where replacement of the native Ser with Ala resulted in a dramatic potency increase. Further potency enhancements are observed when short, straight-chain (Abu) or β-branched (Val, Bug) residues are introduced. Side chains containing more than three saturated carbon atoms in a straight chain are poorly tolerated.

Example 14: Substitutions of Ala^{153} residue in p21(152-159)Ser153Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity: Kinase Inhibition[c]	Relative Activity: Cyclin A Binding[d]
H- **His- Ala- Lys-Arg- rg-Leu- Ile- Phe** -NH_2	28						1	1
H- His- ***Gly***- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	47	$C_{47}H_{80}N_{18}O_8$	1025.3	1026.8	15.2	98	0.1	0.1
H- His- ***Abu***-Lys-Arg- Arg-Leu- Ile- Phe -NH_2	48	$C_{49}H_{84}N_{18}O_8$	1053.3	1055.2	15.8	100	5	1.3
H- His- ***Nva***-Lys-Arg- Arg-Leu- Ile- Phe -NH_2	49	$C_{50}H_{86}N_{18}O_8$	1067.3	1069.1	16.0	100	<0.1	<0.1
H- His- ***Bug***- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	50	$C_{51}H_{88}N_{18}O_8$	1081.4	1082.7	15.9	100	0.2	1.2
H- His- ***Val***- Lys-Arg- Arg- Leu- Ile- Phe -NH_2	51	$C_{50}H_{86}N_{18}O_8$	1067.3	1068.5	15.9	100	2	1.7
H- His- ***Lle***- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	52	$C_{51}H_{88}N_{18}O_8$	1081.4	1081.9	16.1	100	0.5	0.2
H- His- ***Phg***- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	53	$C_{53}H_{84}N_{18}O_8$	1101.4	1101.8	18.8, 16.1[e]	100	<0.1	<0.1
H- His- ***Phe***- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	54	$C_{54}H_{86}N_{18}O_8$	1115.4	1115.8	16.5	100	0.5	0.2

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)
[b] Vydac218TP54, 1 mL/min, 25°C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 µM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)
[e] Mixture of diastereomers (racemic Fmoc-Phg-OH used)

Example 15: Lys^{154}

Various non-isosteric replacements are tolerated to some extent. A significant potency increase is observed when the conservative Lys-to-Arg replacement is made.

Example 15: Substitutions of Lys[154] residue in p21(152-159)Ser153Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity Kinase Inhibition[c]	Relative Activity Cyclin A Binding[d]
H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- His- Ala- ***Ala***-Arg- Arg-Leu- Ile- Phe -NH_2	56	$C_{45}H_{75}N_{17}O_8$	982.2	983.6	15.6	99	<0.1	0.5
H- His- Ala- ***Nle***-Arg- Arg-Leu- Ile- Phe -NH_2	57	$C_{48}H_{81}N_{17}O_8$	1024.3	1022.9	16.8	97	0.3	0.2
H- His- Ala- ***Abu***-Arg- Arg-Leu- Ile- Phe -NH_2	58	$C_{46}H_{77}N_{17}O_8$	996.2	997.4	16.1	100	0.8	0.2
H- His- Ala- ***Leu***-Arg- Arg-Leu- Ile- Phe -NH_2	59	$C_{48}H_{81}N_{17}O_8$	1024.3	1025.5	16.8	97	0.1	1.4
H- His- Ala- ***Arg***-Arg- Arg-Leu- Ile- Phe -NH_2	60	$C_{48}H_{82}N_{20}O_8$	1067.3	1067.9	15.5	94	5.7	1.5

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)
[b] Vydac218TP54, 1 mL/min, 25 °C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 μM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Example 16: Arg[155]

Only the conservative replacements with Cit and Lys are tolerated to some extent.

Example 16: Substitutions of Arg[155] residue in p21(152-159)Ser153Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity Kinase Inhibition[c]	Relative Activity Cyclin A Binding[d]
H- His- Ala- Lys-Arg- rg-Leu- Ile- Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- His- Ala- Lys-***Ala***- Arg-Leu- Ile- Phe -NH_2	01	$C_{45}H_{75}N_{17}O_8$	945.2	954.9	16.0	95	<0.1	<0.1
H- His- Ala- Lys-***Cit***- Arg-Leu- Ile- Phe -NH_2	62	$C_{48}H_{81}N_{17}O_9$	1040.3	1053.5	12.5	94	0.2	0.2
H- His- Ala- Lys- ***se***- Arg-Leu- Ile- Phe -NH_2	63	$C_{46}H_{77}N_{15}O_9$	984.2	985.9	15.8	100	<0.1	<0.1
H- His- Ala- Lys-***His***- Arg-Leu- Ile- Phe -NH_2	64	$C_{48}H_{77}N_{17}O_8$	1020.2	1022.1	15.4	98	<0.1	<0.1
H- His- Ala- Lys-***Nle***- Arg-Leu- Ile- Phe -NH_2	65	$C_{48}H_{81}N_{15}O_8$	996.3	998.4	18.1	86	<0.1	<0.1
H- His- Ala- Lys- ***In***- Arg-Leu- Ile- Phe -NH_2	66	$C_{47}H_{78}N_{16}O_9$	1011.2	1012.9	15.6	98	<0.1	<0.1
H- His- Ala- Lys-***Lys***- Arg-Leu- Ile- Phe -NH_2	67	$C_{48}H_{82}N_{16}O_8$	1011.3	1011.8	15.3	100	0.8	0.1

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)
[b] Vydac 218TP54, 1 mL/min, 25°C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 μM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Example 17: Arg[156]

This residue was probed with replacements constraining the backbone dihedral angles in different ways (Ala, Pro, Aib, Sar), none of which were tolerated. Partially tolerated replacements with Cit or Ser indicate involvement in H-bonding.

Example 17: Substitutions of Arg[156] residue in p21(152-159)Ser153Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity Kinase Inhibition[c]	Relative Activity Cyclin A Binding[d]
H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- His- Ala- Lys-Arg- ***Ala*** -Leu- Ile- Phe -NH_2	68	$C_{45}H_{75}N_{15}O_8$	954.2	954.9	16.1	100	<0.1	<0.1
H- His- Ala- Lys-Arg- ***Asn*** -Leu- Ile- Phe -NH_2	69	$C_{46}H_{76}N_{16}O_9$	997.2	997.5	15.5	99	<0.1	<0.1
H- His- Ala- Lys-Arg- ***Pro*** -Leu- Ile- Phe -NH_2	70	$C_{47}H_{77}N_{15}O_8$	980.2	980.1	16.3	100	<0.1	<0.1
H- His- Ala- Lys-Arg- ***Ser*** -Leu- Ile- Phe -NH_2	71	$C_{45}H_{75}N_{15}O_9$	970.2	970.2	16.1	100	0.7	0.2
H- His- Ala- Lys-Arg- ***Aib*** -Leu- Ile- Phe -NH_2	72	$C_{46}H_{77}N_{15}O_8$	968.1	968.1	16.7	73	<0.1	<0.1
H- His- Ala- Lys-Arg- ***Sar*** -Leu- Ile- Phe -NH_2	73	$C_{45}H_{75}N_{15}O_8$	954.2	955.2	16.5	100	<0.1	<0.1
H- His- Ala- Lys-Arg- ***Cit*** -Leu- Ile- Phe -NH_2	74	$C_{48}H_{81}N_{17}O_9$	1040.3	1041.42	15.67	100	0.3	n/d

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2
[b] Vydac218TP54, 1 mL/min, 25 °C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 μM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2

Example 18: Leu[157]

This residue is very sensitive to replacement, even with nearly isosteric groups. Only the very conservative Leu-to-Ile replacement was tolerated somewhat.

Example 18: Substitutions of Leu[157] residue in p21(152-159) Ser 153 Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity Kinase Inhibition[c]	Relative Activity Cyclin A Binding[d]
H- His- Ala- Lys-Arg-A rg- Leu- Ile-Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- His- Ala- Lys-Arg- Arg- ***Ala*** -Ile- Phe -NH_2	76	$C_{45}H_{76}N_{18}O_8$	997.2	996.9	13.9	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- ***leu*** -Ile- Phe -NH_2	77	$C_{48}H_{82}N_{18}O_8$	1039.3	1041.0	15.1	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- ***Ile*** -Ile- Phe -NH_2	78	$C_{48}H_{82}N_{18}O_8$	1039.3	1041.1	14.4	100	1.5	0.2
H- His- Ala- Lys-Arg- Arg- ***Val*** -Ile- Phe -NH_2	79	$C_{47}H_{80}N_{18}O_8$	1025.3	1026.2	15.8	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- ***Nle*** -Ile- Phe -NH_2	80	$C_{48}H_{82}N_{18}O_8$	1039.3	1040.2	15.8	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- ***Nva*** -Ile- Phe -NH_2	81	$C_{47}H_{80}N_{18}O_8$	1025.3	1025.0	14.9	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- ***Cha*** -Ile- Phe -NH_2	82	$C_{51}H_{86}N_{18}O_8$	1079.4	1079.2	17.5	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- ***Phe*** -Ile- Phe -NH_2	83	$C_{51}H_{80}N_{18}O_8$	1073.3	1072.7	16.4	100	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- ***1Nap*** -Ile- Phe -NH_2	84	$C_{52}H_{82}N_{18}O_8$	1123.4	1122.5	17.9	100	<0.1	<0.1

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)
[b] Vydac218TP54, 1 mL/min, 25 °C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 μM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Example 19: Ile[158]

All substitutions with aliphatic and aromatic residues were tolerated to some extent.

However, excision of the Ile residue abolished activity. These results indicate that this residue is not crucial for activity but may be important as a spacer group between the flanking Leu and Phe groups.

Example 19: Substitutions of Ile[158] Residue in p21 (152-159) Ser153 Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] [M+H]$^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity Kinase Inhibition[c]	Relative Activity Cyclin A Binding[d]
H- His- Ala- Lys-Arg- rg- Leu- Ile-Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- His- Ala- Lys-Arg- Arg- Leu- ***Ala*** -Phe -NH_2	85	$C_{45}H_{76}N_{18}O_8$	997.2	996.5	13.8	100	0.3	0.8
H- His- Ala- Lys-Arg- Arg- Leu- ***Leu*** -Phe -NH_2	86	$C_{48}H_{82}N_{18}O_8$	1039.3	1038.4	16.1	100	1.2	0.6
H- His- Ala- Lys-Arg- Arg- Leu- ***Val*** -Phe -NH_2	87	$C_{47}H_{80}N_{18}O_8$	1025.3	1024.7	14.9	100	0.8	1.5
H- His- Ala- Lys-Arg- Arg- Leu- ***Nle*** -Phe -NH_2	88	$C_{48}H_{82}N_{18}O_8$	1039.3	1040.3	16.3	100	0.4	0.3
H- His- Ala- Lys-Arg- Arg- Leu- ***Nva*** -Phe -NH_2	89	$C_{47}H_{80}N_{18}O_8$	1025.3	1025.7	15.2	100	0.2	0.6
H- His- Ala- Lys-Arg- Arg- Leu- ***Cha*** -Phe -NH_2	90	$C_{51}H_{86}N_{18}O_8$	1079.4	1080.2	18.4	100	0.3	0.5
H- His- Ala- Lys-Arg- Arg- Leu- ***Phe*** -Phe -NH_2	91	$C_{51}H_{80}N_{18}O_8$	1073.3	1073.9	16.3	100	0.4	0.4
H- His- Ala- Lys-Arg- Arg- Leu- ***1Nap***- Phe -NH_2	92	$C_{55}H_{82}N_{18}O_8$	1123.4	1122.9	18.2	100	0.5	0.5
H- His- Ala- Lys-Arg- Arg- Leu- ***Phe*** -NH_2	93	$C_{42}H_{71}N_{17}O_7$	926.1	924.8	13.8	100	<0.1	<0.1

[a] DE MALDI-TOF MS, +ve mode, $_{\alpha}$-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)
[b] Vydac218TP54, 1 mL/min, 25°C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 $_{\mu}$M
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Example 20: Phe[159]

Only certain replacements with aromatic residues were tolerated. Notably pFPhe substitution resulted in an analogue with enhanced cyclin A-binding affinity.

Example 20: Substitutions of Phe[159] residue in p21(152-159)Ser153Ala

Compound	Sequence ID No.	Formula	M_r	MS[a] [M+H]$^+$	RP-HPLC[b] t_R(min)	Purity (%)	Relative Activity Kinase Inhibition[c]	Cyclin A Binding[d]
H- His- Ala- Lys-Arg- Arg- Leu- Ile- Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***Leu*** -NH_2	95	$C_{45}H_{84}N_{18}O_8$	1005.3	1005.7	14.2	97	0.3	<0.1
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***Cha*** -NH_2	96	$C_{48}H_{88}N_{18}O_8$	1045.3	1045.5	16.9	100	<0.1	0.1
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***Hof*** -NH_2	97	$C_{49}H_{84}N_{18}O_8$	1053.3	1052.8	15.8	96	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***Tyr*** -NH_2	98	$C_{48}H_{82}N_{18}O_9$	1055.3	1054.6	13.3	100	0.3	0.2
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***pFPhe*** -NH_2	99	$C_{48}H_{81}N_{18}O_8$	1057.3	1055.8	16.0	100	1	5
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***FPhe*** -NH_2	100	$C_{48}H_{81}N_{18}O_8$	1057.3	1055.5	16.2	100	0.8	0.8
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***Trp*** -NH_2	101	$C_{50}H_{83}N_{19}O_8$	1078.3	1076.1	15.6	98	0.3	0.1
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***1Nap*** -NH_2	102	$C_{52}H_{84}N_{18}O_8$	1089.3	1090.7	17.8	100	0.2	<0.1
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***2Nap*** -NH_2	103	$C_{52}H_{84}N_{18}O_8$	1089.3	1090.6	18.0	100	1.2	0.7
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***Lys*** -NH_2	104	$C_{45}H_{85}N_{19}O_8$	1020.3	1021.5	11.6	66	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- Leu- Ile - ***Tic*** -NH_2	105	$C_{49}H_{82}N_{18}O_8$	1051.3	1052.3	15.6	91	0.3	<0.1

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)
[b] Vydac218TP54, 1 mL/min, 25 °C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm ;
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 μM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Example 21: Substitutions of Phe^{159} residue in p21(152-159)Ser153Ala with conformationally defined residues

Fmoc-DL-*threo*-Pse-OH

To a solution of H-DL-*threo*-Pse-OH (1 g, 5.5 mmol) in 5 % aq Na_2CO_3 (13 mL, 6 mmol), was added a solution of Fmoc-ONSu (1.7 g, 5 mmol) in THF (13 mL) over a period of 30 min. The mixture was stirred vigorously for 5 h. The solvent was evaporated to dryness *in vacuo*. The residual white solid was dissolved in H_2O (150 mL) and was washed with Et_2O (2 □□100 mL). The aqueous phase was acidified to pH 2 with 0.2 M aq HCl and a precipitate was obtained, which was extracted into EtOAc (2 □□100 mL). The combined extracts were washed with aq $KHSO_4$ and brine, dried ($MgSO_4$) and concentrated *in vacuo* to afford a crude product (1.32 g, 65 %). This was dissolved in the minimum volume of EtOAc and dripped into vigorously stirred hexane to afford, after filtration and drying, the title compound (1.27 g, 63 %). M.p. 107 – 108 °C. TLC (EtOAc/AcOH, 99:1): R_f = 0.27. RP-HPLC (Vydac 218TP54, 1 mL/min, 50 – 100 % MeCN in 0.1 % aq CF_3COOH over 20 min): t_R = 7.2 min. ^{1}H-NMR ($CDCl_3$, 250 MHz), □: 7.75 (2H, d, J = 7.6 Hz, Fmoc aromatic *H*), 7.42-7.49 (2h, M, Fmoc aromatic *H*), 7.27-7.39 (9H, m, aromatic *H*), 5.67 (1H, d, J = 9.0 Hz, N*H*), 5.45 (1H, d, J = 2.4 Hz, C$^{□}$*H*), 4.68 (1H, dd, J = 2.5, 8.8 Hz, C$^{□}$*H*), 4.27 (2H, m, Fmoc CH_2), 4.14 (1H, t, J = 7.1 Hz, Fmoc C*H*); ^{13}C-NMR ($CDCl_3$:d_6-

purified by preparative RP-HPLC.

Psa-containing peptides

A portion of the corresponding Pse-containing peptidyl resin (50 mg, 0.015 mmol, theoretical loading 0.293 mmol/g) was suspended in DMF (1 mL) and treated with Ac_2O (14 μL, 0.15 mmol), Pr^i_2NEt (5 μL, 0.02 mmol) and 4-(*N,N*-dimethylamino)pyridine (0.18 mg, 0.0015 mmol). The mixture was gently stirred at room temperature for 24 h. The resin was filtered, washed successively with DMF, CH_2Cl_2 and MeOH, and *dried in vacuo*. The resin product (50 mg) upon acidolytic treatment gave the crude product. Pure peptides were obtained after purification by preparative RP-HPLC.

Compound	SEQ ID No.	Formula	M_r	MS[a]	RP-HPLC[b]		Relative Activity	
				[M+H]	t_R(min)	Purity (%)	Kinase Inhibition[c]	Cyclin A Binding[d]
H- His- Ala- Lys-Arg-A rg- Leu- Ile- Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- His- Ala- Lys-Arg- Arg- Leu- Ile ***L-Pse*** OH	106	$C_{48}H_{82}N_{18}O_9$	1055.9	1055.7	8.8	99	<0.1	0.2
H- His- Ala- Lys-Arg- Arg- Leu- Ile ***D-Pse*** OH	107	$C_{48}H_{82}N_{18}O_9$	1055.9	1056.0	6.8	99	<0.1	0.1
H- His- ***Ser***- Lys-Arg- Arg- Leu- Ile ***L-Pse*** OH	108	$C_{48}H_{82}N_{18}O_{10}$	1071.3	1074.1	8.8	99	<0.1	<0.1
H- His- ***Ser***- Lys-Arg- Arg- Leu- Ile ***D-Pse*** OH	109	$C_{48}H_{82}N_{18}O_{10}$	1071.3	1073.0	6.8	99	<0.1	<0.1
H- His- Ala- Lys-Arg- Arg- Leu- Ile ***L-Psa*** OH	110	$C_{50}H_{84}N_{18}O_{10}$	1097.3	1098.0	11.2	99	22	n/d
H- His- Ala- Lys-Arg- Arg- Leu- Ile ***D-Psa*** OH	111	$C_{50}H_{84}N_{18}O_{10}$	1097.3	1098.0	8.4	99	<0.1	n/d
H- His- ***Ser***- Lys-Arg- Arg- Leu- Ile ***L-Psa*** OH	112	$C_{50}H_{84}N_{18}O_{11}$	1113.3	1114.9	10.8	99	<0.1	n/d
H- His- ***Ser***- Lys-Arg- Arg- Leu- Ile ***D-Psa*** OH	113	$C_{50}H_{84}N_{18}O_{11}$	1113.3	1114.4	8	99	<0.1	n/d
H- His- Ala- Lys-Arg- Arg- Leu- Ile ***Dhp*** OH	114	$C_{48}H_{80}N_{18}O_8$	1037.3	1038.4	8.8	99	3.3	0.2
H- His- ***Ser***- Lys-Arg- Arg- Leu- Ile ***Dhp*** OH	115	$C_{48}H_{80}N_{18}O_9$	1053.3	1054.6	8.8	99	0.4	n/d
H- His- Ala- Lys-Arg- Arg- Leu- Ile ***Pheol***	116	$C_{48}H_{83}N_{17}O_8$	1026.3	1026.2	8.4	99	0.6	1.0
H- His- ***Ser***- Lys-Arg- Arg- Leu- Ile ***Pheol***	117	$C_{48}H_{83}N_{17}O_9$	1042.3	1041.6	8.4	95	0.2	<0.1

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu-Ile- Phe -NH_2 (Seq. ID No. 28)

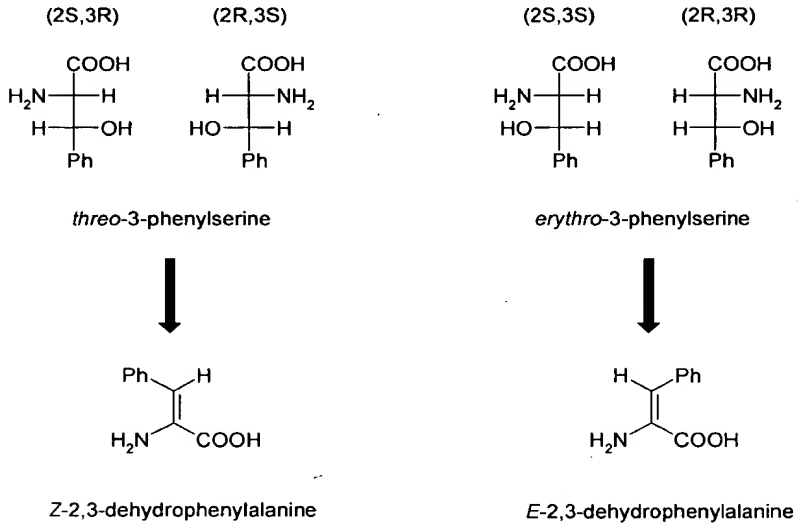
[b] Vydac218TP54, 1 mL/min, 25 °C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm

[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 μM

[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

As is clear from the results presented above, the Phe^{159} residue represents a key determinant in the p21(152-159) pharmacophore: its truncation abolishes activity and certain well-defined substitutions lead to enhanced potency. For this reason, further constriction of the Phe aromatic side chain may lock it into a bio-active conformation and further potency gains may be expected. Such conformational definition can be introduced in many different ways, *e.g.* through further substitution at C^β (as in Psa and Pse), introduction of unsaturation, particularly between C^α and C^β (as in Dhp), or by tethering of the aromatic system to the peptide backbone (C^α and NH), as *e.g.* in Tic (refer

configuration of phenylserine, under basic conditions, eliminated SO_2 stereospecifically to yield the corresponding *Z*-Dhp isomer. Peptides H-His-Ser(Ala)-Lys-Arg-Arg-Leu-Ile-Dhp-OH (SEQ ID Nos. 114 and 115) were typically obtained in > 30 % purity when analysed by RP-HPLC and purified yields of 20-30 %. Conversely, *E*-Dhp peptides would be obtained by analogous dehydration of *erythro*-Pse peptides. Protected Pse peptidyl resins were acetylated selectively at the free hydroxyl of the Pse residue to afford the corresponding *O*-acetylphenylserine (Psa) peptides.



Stereochemistry of 3-phenylserine. The *cis* (*Z*) and *trans* (*E*) isomers of dehydrophenylalanine are derived from *threo*- and *erythro*-phenylserine, respectively, by dehydration.

As far as biological activity is concerned, only the L-Pse/Psa p21(152-159) peptides were able to inhibit CDK2/cyclin A and/or to bind efficiently to cyclin A. Of these, H-His-Ala-Lys-Arg-Arg-Leu-Ile-[*L-Psa*]-OH (SEQ ID No. 110) was particularly potent. Both *Z*-Dhp peptides were biologically active; the Ala^{153} analogue being more potent then the corresponding Ser^{153} peptide. Furthermore, the terminal Phe residue in the p21(152-159) peptides was also replaced with phenylalaninol (Pheol). This substitution was comparatively well-tolerated, showing that the terminal peptide carboxamide (or carboxylate) is not essential in terms of

biological activity.

Example 22: Multiple substitutions in p21(152-159)Ser153Ala,Phe159pFPhe

It was seen above that certain residue substitution in the p21(152-159) peptides were in fact tolerated, and, in some cases, led to increased potency. Some of these single substitutions were then combined in order to test if combinatorial modifications at various positions in the peptide would be additive and/or synergistic. The results suggest that some synergysm is obtained. *E.g.*, combination of His152Ala and Phe159pFPhe replacements yielded a peptide analogue with about 80-fold increased potency, whereas the same substitutions individually lead to 2.5- and 5-fold potency increase (in terms of cyclin A binding) only. Thus, combination of the His152Ala, Ser153Ala, and Phe159pFPhe modifications permitted introduction of *e.g.* Lys154Abu, Arg155Gln, Arg156Cit, Arg156Ser, and Ile158Ala.

Example 22: Multiple substitutions in p21(152-159)Ser153Ala,Phe159pFPhe

Compound	SEQ ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	RP-HPLC[b] Purity (%)	Relative Activity Kinase Inhibition[c]	Relative Activity Cyclin A Binding[d]
H- **His- Ala- Lys-Arg- Arg- Leu- Ile- Phe** -NH_2	28	$C_{48}H_{82}N_{18}O_8$					1	1
H- ***Ala***- Ala- ***Abu***-Arg- Arg- Leu- Ile- ***pFPhe*** -NH_2	118	$C_{43}H_{74}N_{15}O_8F$	948.15	948.16	18.8	99	60	n/d
H- ***Ala***- Ala- Lys-Arg- Arg- Leu- Ile- ***pFPhe*** -NH_2	119	$C_{42}H_{72}N_{13}O_9F$	922.11	922.11	17.82	99	80	n/d
H- ***Ala***- Ala- Lys-Arg- ***Cit***- Leu- Ile- ***pFPhe*** -NH_2	120	$C_{45}H_{78}N_{15}O_9F$	992.2	922.2	16.94	99	10	n/d
H- ***Ala***- Ala- Lys-Arg- Arg- Leu- ***Ala***- ***pFPhe*** -NH_2	121	$C_{42}H_{73}N_{16}O_8F$	949.14	949.69	17.89	99	20	n/d
H- ***Ala***- Ala- ***Abu***-Arg- ***Ser***- Leu- Ile- ***pFPhe*** -NH_2	122	$C_{40}H_{67}N_{12}O_9F$	879.04	879.05	16.56	99	14	n/d
H- ***Ala***- Ala- Lys-***Gln***- Arg- Leu- Ile- ***pFPhe*** -NH_2	123	$C_{44}H_{75}N_{14}O_9F$	963.16	963.17	20.16	99	4	n/d
H- Ala- Lys-Arg- Arg- Leu- Ile- ***pFPhe*** -NH_2	183	$C_{42}H_{75}N_{15}O_7F$	902.15	920.14	16.6	99	4	n/d

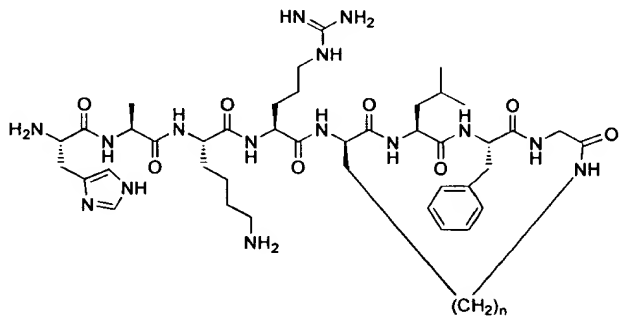
[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)
[b] Vydac218TP54, 1 mL/min, 25 °C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min, λ = 214 nm
[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 μM
[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Example 23: Cyclic peptides

Inspection of the appropriate contacts in the complex structure of cyclin A with a $p27^{KIP1}$ fragment (Russo, A. A.; Jeffrey, P. D.; Patten, A. K.; Massague, J.; Pavletich, N. P. *Nature* **1996**, *382*, 325-31).; suggested a starting point for the design of such conformationally constrained peptides. Asn^{31} of the p27 sequence apparently participates in H-bonds not

peptide synthesis methods. The diamino acid residue which is to participate in the prospective cyclic lactam bond is introduced in an orthogonally protected form, *e.g.* using an Fmoc-diamino acid derivative bearing a side-chain Mtt amino protecting group. After complete chain assembly, the sulfonamide linker is activated through alkylation with iodoacetonitrile. The Mtt protecting group is then removed under mild acidolytic conditions. Intramolecular attack of the liberated amino group on the activated acyl sulfonamide function then results in liberation of the protected cyclic peptide from the solid phase. It is isolated, fully deprotected using strong acidolysis, and purified. A similar approach has recently been reported for the synthesis of 'head-to-tail' cyclic peptides (Zhang, Z.; Van Aerschot, A.; Hendrix, C.; Busson, R.; David, F.; Sandra, P.; Herdewijn, P. *Tetrahedron* **2000**, *56*, 2513-2522). 'Side chain-to-tail' cyclic peptides can be obtained through various known methods, using either solid phase- (refer, *e.g.*, Mihara, H.; Yamabe, S.; Niidome, T.; Aoyagi, H. *Tetrahedron Lett.* **1995**, *36*, 4837-4840) or solution methods (refer, *e.g.*, He, J. X.; Cody, W. L.; Doherty, A. M. *Lett.Peptide Sci.* **1994**, *1*, 25-30).

Using the above method, the peptides 5,8-*cyclo*-[H-His-Ala-Lys-Arg-Lys-Leu-Phe-Gly] (SEQ ID No. 169) and 5,8-*cyclo*-[H-His-Ala-Lys-Arg-Orn-Leu-Phe-Gly] (SEQ ID No. 170) were then synthesised and characterised. The results clearly show that the cyclic constraint introduced is relevant to the peptide's bioactive conformation. Whereas the analogue containing Lys in position 5 was approximately 2 orders of magnitude less potent than the lead peptide H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No. 28), the corresponding Orn analogue was nearly equipotent with the lead peptide.



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5,8-*cyclo*-[H-His-Ala-Lys-Arg-Lys-Leu-Phe-Gly] (**1**, n = 3) (SEQ ID No. 169)

Fmoc-Gly-OH (0.64 g, 2.16 mmol) and 4-sulfamylbutyryl aminomethylpolystyrene resin (Novabiochem; 0.50 g, nominally 0.54 mmol) were suspended in DMF (4.25 mL), and Pr^i_2NEt (0.56 mL, 3.24 mmol) was added. The mixture was stirred for 20 min. After this time, it was cooled to –23 °C, and PyBOP (1.13 g, 2.16 mmol) was added in one portion. Stirring was continued overnight, and the reaction was allowed to warm to room temperature during that period. The resin was then washed thoroughly with DMF, drained, and treated with 50 % acetic anhydride in CH_2Cl_2 (10 mL) for 1 h. After completion, the resin was washed successively with CH_2Cl_2, DMF, and Et_2O, and was dried.

The linear peptide sequence Boc-His(Boc)-Ala-Lys(Boc)-Arg(Pmc)-Lys(Mtt)-Leu-Phe-Gly (SEQ ID No. 274) was then assembled using an ABI 433A peptide synthesiser, employing standard Fmoc protection strategy chemistry. The final peptidyl resin was washed successively with CH_2Cl_2, DMF, and Et_2O, and was dried. An aliquot (0.49 g) was swelled in NMP (4 mL) and treated with iodoacetonitrile (0.37 mL, 5.0 mmol) and Pr^i_2NEt (0.24 mL, 1.25 mmol) under N_2, for 24 h. After this time, the resin was washed thoroughly with NMP (4 x 5 min), DMF, CH_2Cl_2, and Et_2O, before drying. The Lys^5 Mtt side-chain protecting group was then removed by treatment with 1.5 % CF_3COOH, 3% MeOH in 1,2-dichloroethane (3 × 5 mL, 5 min each), and the resin was then washed with further 1,2-dichloroethane, followed by 20 % Pr^i_2NEt in CH_2Cl_2 and Et_2O. The resin was then dried *in vacuo*.

The activated and Lys^5 side chain-deprotected peptidyl resin (100 mg) was swelled in 1,4-dioxane (2 mL; dried over sodium-benzophenone), and dimethylaminopyridine (10 mg) was added. The mixture was then heated at reflux for 14 h, followed by filtering of the resin, and washing with DMF (2 × 5 mL, 5 min). The combined filtrate and washings were evaporated, and the residue was treated with 2.5% Pr^i_3SiH in CF_3COOH solution for 1 h. The peptide product was collected by precipitation in ice-cold Et_2O, and after washing was dried and fractionated by preparative RP-HPLC (Vydac 218TP1022, 9 mL/min, 13 – 23 % MeCN in 0.1 % aq CF_3COOH over 60 min). Fractions containing pure cyclised peptide

were pooled and lyophilised to afford title compound (2.2 mg, 2.34 μmol, 6.5 % w.r.t. initial resin loading). Anal. RP-HPLC: t_R = 15.4 min (Vydac 218TP54, 1 mL/min, 25 °C, 13 – 23 % MeCN in 0.1 % aq CF_3COOH over 20 min), purity > 99 % (λ = 214 nm). DE MALDI-TOF MS: $[M + H]^+$ = 937.8, $C_{44}H_{71}N_{15}O_8$ requires 938.14 (positive mode, α-cyano-4-hydroxycinnamic acid matrix. The presence of the 5,8-cyclic structure was verified by inspection of appropriate through-space connectivities in the NMR ROESY spectrum of the peptide.

5,8-*cyclo*-[H-His-Ala-Lys-Arg-Orn-Leu-Phe-Gly] (**1**, n = 2) (SEQ ID No. 170)

This compound was prepared in a manner analogous to that described above except that residue position 5 was Orn (Fmoc-Orn(Mtt)-OH was used during chain assembly). A portion of the resin (200mg) was then treated as above, to give the pure title compound (6.3 mg, 6.81 μmol, 8.9 % w.r.t. initial resin loading). Anal. RP-HPLC: t_R = 14.09 min (Vydac 218TP54, 1 mL/min, 25 °C, 15 – 25 % MeCN in 0.1 % aq CF_3COOH over 20 min), purity > 99 % (λ = 214 nm). DE MALDI-TOF MS: $[M + H]^+$ = 926.4, $C_{43}H_{69}N_{15}O_8$ requires 924.11 (positive mode, α-cyano-4-hydroxycinnamic acid matrix. The presence of the 5,8-cyclic structure was verified by inspection of appropriate through-space connectivities in the NMR ROESY spectrum of the peptide.

Compound	SEQ ID No.	[Cyclin A] (μg/mL)	Immobilised Ligand[a]	IC_{50}(μM)	Relative Activity
H- His-Ala- Lys-Arg-Arg-Leu- Ile- Phe $-NH_2$	28	5	HAKRRLIF	.03 ±.01	1
5,8-*cyclo*-[H-His-Ala-Lys-Arg-***Lys***-Leu-Phe-***Gly***]	169	5	HAKRRLIF	11.1 ± 0.7	0.03
5,8-*cyclo*-[H-His-Ala-Lys-Arg-***Orn***-Leu-Phe-***Gly***]	170	5	HAKRRLIF	0.7 ± 0.5	0.5
H- His-Ala- Lys-Arg-Arg-Leu- Ile- Phe $-NH_2$	28	10	HAKRRLIF	0.1 ± 0.05	1
5,8-*cyclo*-[H-His-Ala-Lys-Arg-***Lys***-Leu-Phe-***Gly***]	169	10	HAKRRLIF	16 ± 5	0.06
5,8-*cyclo*-[H-His-Ala-Lys-Arg-***Orn***-Leu-Phe-***Gly***]	170	10	HAKRRLIF	0.4 ± 0.2	0.25
H- His-Ala- Lys-Arg-Arg-Leu- Ile- Phe $-NH_2$	28	5	DFYHSKRRLIFS	0.09 ± 0.02	1
5,8-*cyclo*-[H-His-Ala-Lys-Arg-***Lys***-Leu-Phe-***Gly***]	169	5	DFYHSKRRLIFS	8 ± 1	0.01
5,8-*cyclo*-[H-His-Ala-Lys-Arg-***Orn***-Leu-Phe-***Gly***]	170	5	DFYHSKRRLIFS	0.3 ± 0.2	0.3
H- His-Ala- Lys-Arg-Arg-Leu- Ile- Phe $-NH_2$	28	10	DFYHSKRRLIFS	1.8 ± 0.9	1
5,8-*cyclo*-[H-His-Ala-Lys-Arg-***Lys***-Leu-Phe-***Gly***]	169	10	DFYHSKRRLIFS	22 ± 8	0.08
5,8-*cyclo*-[H-His-Ala-Lys-Arg-***Orn***-Leu-Phe-***Gly***]	170	10	DFYHSKRRLIFS	6 ± 7	0.3

[a] Immobilised ligands HAKRRLIF (SEQ ID No. 35); DFYHSKRRLIFS (SEQ ID No. 4)

Example 24: Further truncated peptides

The following truncated peptides were prepared and screened for competitive cyclin A

binding in accordance with the methods described above. The results demonstrate that *N*-terminally truncated analogues of the 8mer p21-derived peptide H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No. 28), and, to a lesser extent, the p27-derived peptide H-Ser-Ala-Abu-Arg-Arg-Asn-Leu-Phe-Gly-NH_2 (SEQ ID No. 275), retain appreciable cyclin A binding capacity at least down to the *C*-terminal 4mer sequences.

Example 24: Further truncated peptides

Compound	SEQ ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] T_R(min)	RP-HPLC[b] Purity (%)	Relative Activity IC_{50}(μM)	Relative Activity Maximum Inhibition (%)
H- Arg- Leu- Ile- Phe -NH_2	24	$C_{27}H_{46}N_8O_4$	546.71	548.6	15.01 [iii]	99	-	50(at 100 (μM)
H- Arg- Arg- Leu- Ile- Phe -NH_2	25	$C_{33}H_{58}N_{12}O_5$	702.9	704.7	13.35 [iii]	99	5	100
H- Lys-Arg- Arg- Leu- Ile - Phe -NH_2	23	$C_{39}H_{70}N_{14}O_6$	831.07	832.8	12.63 [iii]	99	5	100
H- Ala- Lys-Arg- Arg- Leu- Ile -Phe -NH_2	27	$C_{42}H_{75}N_{15}O_7$	902.15	903.9	12.82[iii]	99	2	100
H- His- Ala- Lys-Arg- Arg- Leu- Ile - Phe -NH_2	28	$C_{48}H_{82}N_{18}O_8$	1039.3	1040.4	12.91 [iii]	99	0.3	100
H- Asn- Leu- Phe-Gly -NH_2	29	$C_{21}H_{32}N_6O_5$	448.52	449.6	18.14[i]	99	-	80(at 100 (μM)
H- Arg- Asn- Leu- Phe-Gly -NH_2	30	$C_{27}H_{44}N_{10}O_6$	604.71	605.2	17.17[i]	99	-	20(at 100 (μM)
H- Abu-Arg- Asn- Leu- Phe-Gly -NH_2	31	$C_{31}H_{51}N_{11}O_7$	689.81	690.9	12.87[ii]	99	-	-
H- Ala- Abu-Arg- Asn- Leu- Phe-Gly -NH_2	32	$C_{34}H_{56}N_{12}O_8$	760.89	761.4	13.61[ii]	99	25	90
H- Ser- Ala- Abu-Arg- Asn- Leu-Phe-Gly -NH_2	33	$C_{37}H_{61}N_{13}O_{10}$	847.97	849.1	14.90[ii]	99	15	100

[a] DE MALDI-TOF MS, +ve mode, $_{\alpha}$-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)

[b] Vydac218TP54, 1 mL/min, 25 °C, 0 -40 % MeCN gradient in 0.1 % aq TFA over 20 min, λ = 214 nm; [i] 20-30%, [ii] 23-33%, [iii] 25-35%

[c] CDK2 / cyclin A kinase assay , pRb substrate, [ATP] = 100 $_{\mu}$M

[d] Competitive cyclin A binding assay using immobilised biotinyl-Ahx-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (Seq. ID No. 192)

Example 25: Peptide analogues of H-Ala-Ala-Lys-Arg-Arg-Leu-Ile-pFphe-NH_2 (SEQ ID No. 119)

Compound	SEQ ID No.	Formula	M_r	MS[a] $[M+H]^+$	RP-HPLC[b] t_R(min)	Purity (%)
H- His- Ala- Lys- Arg- Arg- Leu- Ile- pFPhe -NH_2	119	$C_{45}H_{79}FN_{16}O_8$	991.2	991.1	12.45	90
H- ***Gly***- Ala- Lys- Arg- Arg- Leu- Ile- pFPhe -NH_2	125	$C_{44}H_{77}FN_{16}O_8$	977.2	976.4	15.9	94
H- His- Ala- Lys- ***hArg***- Arg- Leu- Ile- pFPhe -NH_2	126	$C_{46}H_{81}FN_{16}O_8$	1005.3	1004.1	12.47	85
H- His- Ala- Lys- ***Ser***- Arg- Leu- Ile- pFPhe -NH_2	127	$C_{42}H_{72}FN_{13}O_9$	922.1	921.0	12.64	87
H- His- Ala- Lys- ***Hse***- Arg- Leu- Ile- pFPhe -NH_2	128	$C_{43}H_{74}FN_{13}O_9$	936.1	935.5	12.68	87
H- His- Ala- Lys- Arg- ***Lys***- Leu- Ile- pFPhe -NH_2	129	$C_{45}H_{79}FN_{14}O_8$	963.2	962.3	12.24	90
H- His- Ala- Lys- Arg- ***Orn***- Leu- Ile- pFPhe -NH_2	130	$C_{44}H_{77}FN_{14}O_8$	949.2	948.3	12.35	95
H- His- Ala- Lys- Arg- ***Gln***- Leu- Ile- pFPhe -NH_2	131	$C_{44}H_{75}FN_{14}O_9$	963.2	962.6	12.58	93
H- His- Ala- Lys- Arg- ***Hse***- Leu- Ile- pFPhe -NH_2	132	$C_{43}H_{74}FN_{13}O_9$	936.1	934.9	12.83	90
H- His- Ala- Lys- Arg- ***Thr***- Leu- Ile- pFPhe -NH_2	133	$C_{43}H_{74}FN_{13}O_9$	936.1	934.8	12.88	92
H- His- Ala- Lys- Arg- ***Nva***- Leu- Ile- pFPhe -NH_2	134	$C_{44}H_{76}FN_{13}O_8$	934.2	932.6	13.74	93
H- His- Ala- Lys- Arg- Arg- ***Phg***- Ile- pFPhe -NH_2	135	$C_{47}H_{75}FN_{16}O_8$	934.2	1009.8	11.42	90
H- His- Ala- Lys- Arg- Arg- ***Met***- Ile- pFPhe -NH_2	136	$C_{44}H_{77}FN_{16}O_8S$	1011.2	1009.2	12.04	80
H- His- Ala- Lys- Arg- Arg- ***Ala***- Ile- pFPhe -NH_2	137	$C_{42}H_{73}FN_{16}O_8$	1009.3	948.1	11.43	82
H- His- Ala- Lys- Arg- Arg- ***Hof***- Ile- pFPhe -NH_2	138	$C_{49}H_{79}FN_{16}O_8$	949.1	1038.0	13.37	88
H- His- Ala- Lys- Arg- Arg- ***Leu***- Ile- pFPhe -NH_2	119	$C_{46}H_{81}FN_{16}O_8$	1039.3	1003.1	13.2	86
H- His- Ala- Lys- Arg- Arg- alle- Ile- pFPhe -NH_2	140	$C_{45}H_{79}FN_{16}O_8$	1005.3	989.5	12.32	75
H- His- Ala- Lys- Arg- Arg- Leu- ***Gly***- pFPhe -NH_2	141	$C_{41}H_{71}FN_{16}O_8$	991.2	934.6	11.25	84
H- His- Ala- Lys- Arg- Arg- Leu- ***βAla***- pFPhe -NH_2	142	$C_{42}H_{73}FN_{16}O_8$	935.1	947.9	14.3	94
H- His- Ala- Lys- Arg- Arg- Leu- ***Pgh***- pFPhe -NH_2	143	$C_{47}H_{75}FN_{16}O_8$	949.1	1009.7	12.8,14.1	88
H- His- Ala- Lys- Arg- Arg- Leu- ***Aib***- pFPhe -NH_2	144	$C_{43}H_{75}FN_{16}O_8$	1011.2	961.7	15.7	95
H- His- Ala- Lys- Arg- Arg- Leu- ***Sar***- pFPhe -NH_2	145	$C_{42}H_{73}FN_{16}O_8$	963.2	947.8	11.4	87
H- His- Ala- Lys- Arg- Arg- Leu- ***Pro***- pFPhe -NH_2	146	$C_{44}H_{75}FN_{16}O_8$	949.1	973.8	11.9	90
H- His- Ala- Lys- Arg- Arg- Leu- ***Bug***- pFPhe -NH_2	147	$C_{45}H_{79}FN_{16}O_8$	975.2	990.2	15.6	90
H- His- Ala- Lys- Arg- Arg- Leu- ***Ser***- pFPhe -NH_2	148	$C_{42}H_{73}FN_{16}O_9$	965.1	964.4	14.1	85
H- His- Ala- Lys- Arg- Arg- Leu- ***Asp***- pFPhe -NH_2	149	$C_{43}H_{73}FN_{16}O_{10}$	993.2	992.4	14.2	95
H- His- Ala- Lys- Arg- Arg- Leu- ***Asn***- pFPhe -NH_2	150	$C_{43}H_{74}FN_{17}O_9$	992.2	990.5	13.8	94
H- His- Ala- Lys- Arg- Arg- Leu- ***pFPhe***- pFPhe -NH_2	151	$C_{48}H_{77}FN_{16}O_8$	1025.2	1024.1	16.8	94
H- His- Ala- Lys- Arg- Arg- Leu- ***diClPhe***- pFPhe -NH_2	152	$C_{48}H_{76}Cl_2N_{16}O_8$	1076.1	1074.9	18.9	92
H- His- Ala- Lys- Arg- Arg- Leu- ***pClPhe***- pFPhe -NH_2	153	$C_{48}H_{77}ClN_{16}O_8$	1041.7	1041.1	17.8	95
H- His- Ala- Lys- Arg- Arg- Leu- ***mClPhe***- pFPhe -NH_2	154	$C_{48}H_{77}ClN_{16}O_8$	1041.7	1058.1	17.9	95
H- His- Ala- Lys- Arg- Arg- Leu- ***oClPhe***- pFPhe -NH_2	155	$C_{48}H_{77}ClN_{16}O_8$	1041.7	1041.0	17.2	95
H- His- Ala- Lys- Arg- Arg- Leu- ***pIPhe***- pFPhe -NH_2	156	$C_{48}H_{771}N_{16}O_8$	1133.1	1132.6	18.5	95
H- His- Ala- Lys- Arg- Arg- Leu- ***TyreMe***- pFPhe -NH_2	157	$C_{49}H_{80}N_{16}O_8$	1037.3	1036.7	16.4	91
H- His- Ala- Lys- Arg- Arg- Leu- ***Thi***- pFPhe -NH_2	158	$C_{46}H_{76}N_{16}O_8S$	1013.3	1012.7	16.1	95
H- His- Ala- Lys- Arg- Arg- Leu- ***Pya***- pFPhe -NH_2	159	$C_{47}H_{77}N_{17}O_8$	1008.2	1007.1	13.5	86
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***diClPhe*** -NH_2	160	$C_{45}H_{78}Cl_2N_{16}O_8$	1042.1	1005.8	18.6	91
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***pClPhe*** -NH_2	161	$C_{45}H_{79}ClN_{16}O_8$	1007.7	1004.2	17.3	88
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***mClPhe*** -NH_2	162	$C_{45}H_{79}ClN_{16}O_8$	1007.7	1006.8	17.3	88
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***oClPhe*** -NH_2	163	$C_{45}H_{79}ClN_{16}O_8$	1007.7	1007.0	16.5	84
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***Phg*** -NH_2	164	$C_{44}H_{78}N_{16}O_8$	959.2	958.8	14.6,15.8[c]	95
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***TyrMe*** -NH_2	165	$C_{46}H_{82}N_{16}O_9$	1003.3	1002.8	15.7	90
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***Thi*** -NH_2	166	$C_{43}H_{78}N_{16}O_8S$	979.3	978.6	15.1	87
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***Pya*** -NH_2	167	$C_{44}H_{79}N_{17}O_8$	974.2	973.7	11.5	90
H- His- Ala- Lys- Arg- Arg- Leu- Ile- ***Inc*** -NH_2	168	$C_{45}H_{79}FN_{16}O_8$	971.2	(878.99)	16.1	95

[a] DE MALDI-TOF MS, +ve mode, α-cyano-4-hydroxycinnamic acid matrix, calibration on authentic H- His- Ala- Lys-Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)

[b] Vydac 218TP54, 1 mL/min, 25°C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min

[c] Mixture of diastereomers (racemic Fmoc-Phg-OH used)

Example 26: Peptide Analogues of H-Ala-Ala-Lys-Arg-Arg-Leu-Phe-Gly-NH_2 (SEQ ID No. 233)

Compound	SEQ ID No.	Formula	M_r	MS[a] [M+H]$^+$	RP-HPLC[b] t_R(min)	Purity (%)
H- Ala- Ala- Lys- Arg- Arg- Leu- Phe- Gly -NH_2	233	$C_{41}H_{72}N_{16}O_8$	917.1	916.1	13.7	94
H- Ala- Ala- Lys- ***hArg***- Arg- Leu- Phe- Gly -NH_2	234	$C_{42}H_{74}N_{16}O_8$	931.2	929.4	13.8	93
H- Ala- Ala- Lys- ***Ser***- Arg- Leu- Phe- Gly -NH_2	235	$C_{38}H_{65}N_{13}O_9$	848.0	847.4	14.1	95
H- Ala- Ala- Lys- ***Hse***- Arg- Leu- Phe- Gly -NH_2	236	$C_{39}H_{67}N_{13}O_9$	862.0	861.1	13.9	90
H- Ala- Ala- Lys- Arg- ***Lys***- Leu- Phe- Gly -NH_2	237	$C_{41}H_{72}N_{14}O_8$	889.1	888.8	13.5	90
H- Ala- Ala- Lys- Arg- ***Orn***- Leu- Phe- Gly -NH_2	238	$C_{40}H_{70}N_{14}O_8$	875.1	874.6	13.5	95
H- Ala- Ala- Lys- Arg- ***Gln***- Leu- Phe- Gly -NH_2	239	$C_{40}H_{68}N_{14}O_9$	889.1	887.7	13.7	86
H- Ala- Ala- Lys- Arg- ***Hse***- Leu- Phe- Gly -NH_2	240	$C_{39}H_{67}N_{13}O_9$	862.0	861.3	13.9	88
H- Ala- Ala- Lys- Arg- ***Thr***- Leu- Phe- Gly -NH_2	241	$C_{39}H_{67}N_{13}O_9$	862.0	860.4	14.3	90
H- Ala- Ala- Lys- Arg- ***Nva***- Leu- Phe- Gly-NH_2	242	$C_{40}H_{69}N_{13}O_8$	860.1	858.7	15.6	85
H- Ala- Ala- Lys- Arg- Arg- ***Met***- Phe- Gly-NH_2	243	$C_{40}H_{70}N_{16}O_8$ S	935.2	934.1	10.9	93
H- Ala- Ala- Lys- Arg- Arg- ***Ala***- Phe- Gly-NH_2	244	$C_{38}H_{66}N_{16}O_8$	875.0	872.2	12.7	95
H- Ala- Ala- Lys- Arg- Arg- ***Hof***- Phe- Gly-NH_2	245	$C_{45}H_{72}N_{16}O_8$	965.2	962.9	15.1	81
H- Ala- Ala- Lys- Arg- Arg- ***Hle***- Phe- Gly-NH_2	246	$C_{42}H_{74}N_{16}O_8$	931.2	930.1	15.2	94
H- Ala- Ala- Lys- Arg- Arg- ***alle***- Phe- Gly-NH_2	247	$C_{41}H_{72}N_{16}O_8$	917.1	915.9	13.2	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***Tic***- Gly -NH_2	248	$C_{42}H_{72}N_{16}O_8$	929.1	928.3	13.7	93
H- Ala- Ala- Lys- Arg- Arg- Leu- ***Pgh***- Gly -NH_2	249	$C_{40}H_{70}N_{16}O_8$	903.1	902.0	12.3,13.7[c]	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***pFPhe***-Gly-NH_2	250	$C_{41}H_{71}FN_{16}O_8$	935.1	933.7	14.3	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***pIPhe***- Gly-NH_2	251	$C_{41}H_{71}IN_{16}O_8$	1043.0	1041.3	16.4	92
H- Ala- Ala- Lys- Arg- Arg- Leu- ***Thi***- Gly-NH_2	252	$C_{39}H_{70}N_{16}O_8$ S	923.2	920.8	13.2	96
H- Ala- Ala- Lys- Arg- Arg- Leu- ***Pya***- Gly-NH_2	253	$C_{40}H_{71}N_{17}O_8$	918.1	915.1	9.3	90
H- Ala- Ala- Lys- Arg- Arg- Leu- ***diClPhe***-Gly-NH_2	254	$C_{41}H_{70}Cl_2N_{16}O_8$	986.0	984.2	17	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***pClPhe***-Gly -NH_2	255	$C_{41}H_{71}ClN_{16}O_8$	951.6	950.2	15.5	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***mClPhe***-Gly-NH_2	256	$C_{41}H_{71}ClN_{16}O_8$	951.6	949.8	15.5	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***oClPhe***- Gly-NH_2	257	$C_{41}H_{71}ClN_{16}O_8$	951.6	949.9	15	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***1Nap***- Gly-NH_2	258	$C_{45}H_{74}N_{16}O_8$	967.2	965.7	16.3	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***2Nap***- Gly-NH_2	259	$C_{45}H_{74}N_{16}O_8$	967.2	966.1	16.4	95
H- Ala- Ala- Lys- Arg- Arg- Leu- ***Inc***- Gly-NH_2	260	$C_{41}H_{70}N_{16}O_8$	915.1	917.8	14.36	90
H- Ala- Ala- Lys- Arg- Arg- Leu- Phe- ***Asp***-NH_2	261	$C_{43}H_{74}N_{16}O_{10}$	975.2	972.5	13.6	95
H- Ala- Ala- Lys- Arg- Arg- Leu- Phe- ***Glu***-NH_2	262	$C_{44}H_{76}N_{16}O_{10}$	989.2	987.5	13.3	93
H- Ala- Ala- Lys- Arg- Arg- Leu- Phe- ***Ser***-NH_2	263	$C_{42}H_{74}N_{16}O_9$	947.2	944.7	13.1	95
H- Ala- Ala- Lys- Arg- Arg- Leu- Phe- ***Asn***-NH_2	264	$C_{43}H_{75}N_{17}O_9$	974.2	972.6	13.3	95
H- Ala- Ala- Lys- Arg- Arg- Leu- Phe- ***Gln***-NH_2	265	$C_{44}H_{77}N_{17}O_9$	988.2	986.9	12.5	95
H- Ala- Ala- Lys- Arg- Arg- Leu- Phe- ***Lys***-NH_2	266	$C_{45}H_{81}N_{17}O_8$	988.2	987.0	13.6	95

[a] DE MALDI-TOF MS, +ve mode, $_{\alpha}$-cyan-4-hydroxycinnamic acid matrix, calibration on authentic H- Ala- Ala- Lys- Arg- Arg-Leu- Ile- Phe -NH_2 (Seq. ID No. 28)

[b] Vydac 218TP54, 1 mL/min, 25°C, 0 -40 % MeCN in 0.1 % aq TFA over 20 min

[c] Mixture of diastereomers (racemic Fmoc-Phg-OH used)

Example 27 :ASSAYS

Example of a cyclin affinity capture method for the identification of peptide inhibitors

Peptides were synthesized as described above. Cyclin D1 was expressed in E coli BL21(DE3) using PET expression vector and purified from the inclusion bodies. After refolding Cyclin D1 was cross-linked on SulfoLink agarose support (PIERCE). CDK4-6 x His was expressed in Sf9 insect cells infected with the appropriate baculovirus construct and purified by metal-affinity chromatography (Quiagen). GST-Rb (773-924) was expressed in E coli and purified on a Glutathione-Sepharose column according the manufacturers instructions (Pharmacia). CDK4/Cyclin D1 phosphorilation of Rb was determined by incorporation of radio-labeled phosphate in GST-Rb in 96-well format kinase assay. The phosphorylation reaction mixture consisted of 50 mM HEPES pH 7.4, 20 mM $MgCl_2$, 5 mM EDTA, 2 mM DTT, 20 mM -glicerophosphate, 2 mM NaF, 1 mM Na_3VO_4, 0.5 g CDK4, 0.5 g Cyclin D1, 10 l GST-Rb Sepharose beads, 100 M ATP and 0.2 Ci ^{32}P-ATP. The reaction was carried out for 30 min at 30 C at constant shaking. The GST-Rb-Sepharose beads were washed with 50 mM HEPES and 1 mM ATP and the radioactivity was measured on Scintillation counter (Topcount, HP)

Three Dimensional Models

As described in Example 4 above, a computer generated model of a preferred peptide of the present invention (HAKRRLIF) (SEQ ID No. 35) complexed to cyclin A has been generated using AFFINITY (Molecular Simulations Inc.). A representation of this complex is shown in Figure 4. Using the bond dimension analysis the following cyclin A amino acids have been determined as important in forming associations with this peptide:

protein - protein interactions to be delineated in terms of side-chain and backbone flexibility and using a routine employing full molecular mechanics description of non-bonded interactions. The generated model (***Fig. 4***) gives additional understanding of the molecular basis of the affinity of the peptide for the cyclin groove since it reveals that the residues that are intolerant to substitution and deletion make important contacts with the protein.

As with Examples 12-22, the following discussion relates to observations made in respect of the peptide HAKRRLIF (SEQ ID No. 35) and all conclusions drawn in respect of potency increasing or decreasing are to be so interpreted. Two immediate conclusions can be drawn from the structure regarding the explanation of the functional significance of residues and which cannot be readily made from the available experimental data. The first is the rationale for the significant potency increase observed in the Ser153Ala substitution and the second is the accommodation of an aromatic residue in either position 7 or 8 of the cyclin binding motif (position 7 in conjunction with Gly at position 8). The basis for this can be ascertained by comparing the X-ray structure of the $p27^{KIP1}$ ternary complex with the binary docked model structure. For the interaction of the LFG motif in the p27 structure, the Leu and Phe residues insert into the hydrophobic pocket formed by Met^{210}, Ile^{213}, Trp^{217}, and Leu^{253} provide the majority of the binding interaction of this region with the cyclin molecule. For the interactions of the LIF motif, the backbone torsion angles of the peptide at positions 6, 7 and 8 adjust in order to allow the Phe side chain to rotate into the hydrophobic pocket and form a high degree of complementarity with the hydrophobic pocket residue of the groove. The Ile side chain at position 7 (158 of p21) rotates out of the pocket to accommodate the Phe and no longer makes any hydrophobic contacts (see ***Fig. 5***). The conformational changes that the peptide undergoes relative to the p27 structure in order to adapt the position 8 Phe residue into the hydrophobic pocket are quite marked. The comparison of the bound peptide structures in ***Fig. 5*** illustrates how the turn structure on the NLFG (SEQ ID No. 29) sequence in p27 which forms both intra- and inter-molecular hydrogen bonds is no longer present in the p21 peptide structure and is replaced by a more extended backbone conformation.

This observation explains the ability of the spacer residue between the Leu and Phe not only to be tolerated but also to increase affinity significantly as suggested by the observation that HAKRRLIF (SEQ ID No. 35) is more potent than is the hybrid peptide HAKRRLFG (SEQ ID No. 37). The ability of position 7 analogues including Ala to retain binding with cyclin A also supports this conclusion. The second observation and explanation that can be extracted from the model is the reason for the ability of the Ala replacement at position 153 dramatically to increase binding. This residue in the model forms hydrophobic contact with a second minor pocket which is made up by the second face of the Trp involved in the major pocket and two other residues. In the docked model, this second minor pocket is more pronounced and forms more complementary interactions with Ala than is observed in the crystal structure. It is apparent from this site that placement of the polar Ser residue in this hydrophobic environment would not be favoured and in fact would destabilise the binding interaction of the p21 peptide for the cyclin.

Further examination of the cyclin-bound p21 complex gives further indications of the nature of the residues that contribute to the affinity of the peptide to the recruitment site and that are different to those in the cyclin binding motif of p27. These include the His at position1 (Ser^{27} in p27), Lys at position 2 (Cys), and Arg at position 5 (Asn). The Ser to His change from p27 to p21 does not appear to be a critical one since both the Ala replacement peptide (p21(149-160)His152Ala) and the truncated peptide minus the residue at position 1 are essentially equipotent. This result is consistent with the binding model since this residue does not form any contacts with the protein with the exception of an H-bond donation of the terminal amino group. By contrast of the Cys to Lys variant, functional data indicates that the Ala mutant undergoes a two-fold reduction in its ability to phosphorylate pRb. From the calculated model, Lys^{154} forms an ion pair interaction with Asp^{284} thus suggesting the basis for the potency decrease with this residue. Finally the Asn to Arg (156 in p21) change leads to a six-fold reduction in potency suggesting that the guanidino function of position 5 contributes to the binding interaction. Again the model indicates that this residue plays an important role in forming hydrogen bonds

corresponding to those observed to the Asn residue in the p27 structure and thereby contributing to validation of the docked model. In addition, the recently published structure of a p107 peptide bound to cyclin A verifies the model since the homologous Arg in this structure H-bonds to Asp^{283}, an interaction which is also observed in the docked complex (Brown, N. R.; Noble, M. E.; Endicott, J. A.; Johnson, L. N. *Nat. Cell Biol.* **1999**, *1*, 438-443).

Other than those interactions identified as being unique to the peptides of the present invention, there are the residues that are conserved between p27 and the p21 *C*-terminally optimised peptides that form similar interactions to those observed in the experimentally derived structure. In particular, Arg^{155}, forms H-bonding and electrostatic interactions with Asp^{216} and Glu^{200} and Leu^{157} of the hydrophobic motif inserts into the pocket in a similar orientation to that observed in the crystal structure.

In summary, the model structure of the potent CDK2 and CDK4 inhibitor peptide H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No. 28) in complex with CDK2/cyclin A gives considerable insight into the intermolecular interactions involved in cyclin binding and hence into blocking of substrate recruitment. In conjunction with kinase activity data for the series of p21 truncation and substitution analogues, this model clearly defines the sequence and structural requirements of the cyclin binding motif.

The $pFPhe^8$ derivative of the peptide H-His-Ala-Lys-Arg-Arg-Leu-Ile-Phe-NH_2 (SEQ ID No. 28) was found to possess increased activity in binding assays with cyclin A. Molecular modelling docking simulations performed with this analogue (***Fig. 6***) suggested that the pFPhe derivative inserts deeper into the hydrophobic pocket of the cyclin groove. This appears to result from rearrangement of the residues of the pocket forming more complementary interactions with the pFPhe residue and probably results from the change in charge distribution of the ring relative to the unsubstituted amino acid. This apparent gain in peptide-receptor affinity due to improved hydrophobic interactions of the pFPhe residue suggests that reduction of molecular mass through further *N*-terminal truncation will be